# Currents in Nutrition

Proceedings of the Nutrition Symposium held at The University of Illinois, College of Medicine, November 19, 1949

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June, 1950



THE NATIONAL VITAMIN FOUNDATION, INCORPORATED
150 Broadway, New York 7, New York

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Price $1.00

# Contents

## Foreword

This volume was prepared largely from the stenotypist's transcript of the actual proceedings of the nutrition symposium at the University of Illinois, November 19, 1949. In all instances, therefore, the addresses are published without bibliographies. The timeliness and importance of the work presented, however, as well as the professional competence of the speakers, well compensates for the lack of tables of references to the literature and the reader, be he in clinical research or in the practice of medicine, will find these proceedings interesting and of value.

All who participated in the conference are grateful to Dr. Otto A. Bessey and his Committee at the University of Illinois, College of Medicine, who were responsible for the program and the arrangements.

ROBERT S. GOODHART, M.D.
*Editor.*

## Introduction

The opening session of the Symposium on Currents in Nutrition, at the University of Illinois, College of Medicine, Chicago, Illinois, convened at 9:15 a. m., November 19, 1949, Dr. John B. Youmans, Dean, College of Medicine, University of Illinois, presiding.

CHAIRMAN YOUMANS: I am very happy personally, as well as a representative of the University and of the College of Medicine, to welcome you this morning. We are both pleased and honored to be host to such a distinguished group and to participate in the excellent program which has been arranged by the head of our Department of Biochemistry, Dr. Otto A. Bessey, through the generosity of The National Vitamin Foundation.

Those of us who are interested in nutrition are very grateful to the Foundation for the fine contribution they have made in the way of these Symposia as well as in other ways, and I am sure all of us this morning are happy at the opportunity to participate in another of them.

Our first topic this morning is, "Studies on Thiamine and Riboflavin Requirements in Man," by Dr. M. K. Horwitt, of the Elgin State Hospital. Dr. Horwitt:

# STUDIES ON THIAMINE AND RIBOFLAVIN REQUIREMENTS IN MAN

M. K. HORWITT
Chief, Biochemical Research Laboratory, Elgin State Hospital, Elgin, Illinois.

Dr. Youmans, Ladies and Gentlemen: May I begin by thanking the National Vitamin Foundation, its officers, and the committee in charge of these proceedings for the opportunity they have given me to present the results of our research.

Our nutritional research of the past six years has been divided into two parts; the first was concerned with a study of human thiamine and riboflavin requirements, with emphasis on the thiamine requirement; the second, was devoted exclusively to a study of riboflavin.

In the first project, 36 male patients were initially divided into three groups designated as groups A, B and C, respectively. Each person in the A group received a daily diet containing approximately 2200 calories and apparently adequate in all essentials except thiamine and riboflavin. It contained approximately 0.4 mg. of thiamine and 0.85 mg. of riboflavin. The subjects in the B group received the same diet plus a daily supplement of a special yeast extract containing approximately 6 mg. of thiamine and 1.3 mg. of riboflavin. Members of the C group received the regular hospital diet containing about 1.0 mg. of thiamine and 1.6 mg. of riboflavin. The clinical effect of the moderate restriction of thiamine and riboflavin on subjects of the A group, who received a total of 0.4 mg.

thiamine and 0.85 mg. riboflavin daily, was at no time more than minimal, even though the majority remained on the regimen for over two and a half years.

Although some changes were obtained in the lactic and pyruvic acid levels of the blood, the debatable nature of the clinical changes observed on 0.4 mg. of thiamine daily stimulated a plan to feed a diet containing less thiamine. Therefore, at the beginning of the third year, while the A group continued on the diet containing 0.4 mg. of thiamine, the B group, which had been receiving the same diet plus a yeast supplement, was placed on a diet more rigidly restricted in thiamine. This latter diet contained 0.2 mg. of thiamine and 0.75 mg. of riboflavin, one half as much thiamine and about the same amount of riboflavin as the A group continued to receive. Incontrovertible manifestations of thiamine deficiency appeared in all of the B group within six months. Among the circulatory effects was a non-pitting swelling of the facial skin, and an edema of the lower extremities. A loss of achilles and patellar tendon reflexes was accompanied by pronounced difficulties in locomotion.

The vibration sense in the lower extremities decreased as the nutritional deficiency progressed. There was a pronounced loss of appetite, accompanied by a feeling of nausea, in some subjects. The mental changes were sudden in onset and quite severe in character. Previous psychotic patterns were reinforced or reawakened and there was a more explosive onset of tantrums, furors and rages than had previously been evident. This was not shown by the subjects in the other groups.

The therapeutic response to vitamin supplementation was dramatic, especially in the subjective fields

of appetite, general feeling, pain and paresthesia. Edema of the ankles disappeared overnight. Lost patellar reflexes began to return soon after supplementation. On the other hand, the achilles tendon reflex was slow to return, taking as long as a year in one case.

The many papers that followed the early demonstrations that lactic and pyruvic acids increase in the tissues of thiamine-deficient animals provide ample proof that blood pyruvic acid is elevated during thiamine deficiency; however, the use of basal levels of pyruvic acid as a means of determining early thiamine deficiency has not proven satisfactory.

Bueding, Stein and Wortis, and Williams, Mason, Smith and Wilder, compared the blood pyruvate levels obtained after glucose ingestion in normal and thiamine-deficient subjects and showed that the ingestion of glucose causes a temporary but significant elevation of blood pyruvic acid which is abnormally prolonged and elevated during thiamine deficiency. Both groups of workers concluded that a "metabolic load", such as glucose ingestion, provides more reliable data than does the basal levels of pyruvic acid.

Accordingly, when our project started, the technique of administering 1.8 grams of glucose per kilogram of body weight and then determining blood levels of glucose, lactic and pyruvic acids at 60, 120 and 180 minutes was included as a regular part of our laboratory procedure. The average lactic and pyruvic acid results obtained showed distinct differences between the A group on 0.4 mg. of thiamine and the B group (supplemented), within three months after the diets were started. However, there were individual cases in which subjects receiving a thiamine supple-

ment had higher lactic and pyruvic acid levels than did those on the deficient diet. This was found to be due to the fact that the glucose tolerance curves varied markedly, and the blood levels of lactic and pyruvic acid tended to remain proportional to the glucose level in the ratio of 100 to 10 to 1 for glucose, lactic acid and pyruvic acid, respectively. This fact has been documented elsewhere and will not be described in detail here but it can be seen that, since no two people need have the same glucose level 60 minutes after glucose ingestion, one cannot compare their blood levels of lactic acid or of pyruvic acid at that time, without also considering the blood level of glucose.

The recognition of the importance of the load led us to consider the possibility of adding to the metabolic load by exercising the subjects at the time when the glucose levels were at a peak. (The exercise has to be relatively mild in order to avoid a state of anoxia where cardiovascular efficiency would be tested, rather than cellular metabolism). A simple stair climbing test proved satisfactory. Immediately after the 60 minute blood sample was drawn, the patient was walked up and down a flight of stairs twice in one minute. A blood sample was drawn five minutes later, at which time the average blood lactic and pyruvic acids were found to be at maximum levels for the amount of exercise indicated. This blood sample was called the 66 minute sample.

The correlation of hundreds of data showed that the ratio of lactic acid to pyruvic acid in the blood at 5 minutes after the completion of the amount of exercise described, was approximately 15 to 1. It was therefore possible to develop a simple arithmetical ex-

pression to relate the values of glucose, lactic and pyruvic acid—thus,

$$CMI = \frac{\frac{L\text{-}G}{10} + \frac{15P\text{-}G}{10}}{2}$$

A demonstration of the correlation of chemical changes with clinical state is given in the following typical case: During the period of supplementation, normal levels for the index of carbohydrate metabolism as well as for reflex responses were obtained. Within 3 months after the start of feeding of the depleted diet the index of carbohydrate metabolism (CMI) rose to a figure above 15. Two months later there was evidence of loss of the achilles tendon reflex which disappeared completely a month later. Supplementation rapidly reversed these effects, but the achilles tendon reflex took a long time to return to normal. Similar data on all subjects, many of which were more striking than the example chosen, have recently been published.

Subjects in the A group, on the diet providing 0.4 mg. of thiamine per day, fluctuated above and below the critical level of 15, indicating that, although there may have been pathology present, it was not so bad that some could not recover spontaneously from time to time. Every subject in the B group had indices below 15 during the period when they were on the supplemented diet, but, when they were placed on a diet containing only half as much thiamine as the A group (June 1945), every subject soon rose to levels above 15. The curve for group C, which ate the regular hospital diet, remained in a constant narrow range throughout the experiment. The recoveries after supplementation of both the A and B group were very dramatic.

The second project was an attempt to answer the following questions which had been raised by conflicts in the nutritional literature. Can a pure riboflavin deficiency be produced experimentally? If skin lesions are observed during subsistence on diets low in riboflavin are these lesions specific signs of riboflavin deficiency? And can biochemical methods be devised to spot riboflavin deficiency before it becomes clinically obvious?

The choice of the level of riboflavin fed, was influenced by the positive results reported by Sebrell and Butler in 1938 on a diet that contained 0.5 mg. per day and the negative results of more recent workers, Williams, Mason, Cusick and Wilder, and Keys, Henschel, Mickelson, Brozek and Crawford and others, who fed larger amounts.

The composition of the diet, the choice and division of subjects into groups was similar to that used in the previous project. Group A, 15 subjects, received a diet supplying 0.55 mg. of riboflavin per day, Group B, 14 subjects, received the same diet but in addition were given a tablet each day containing 2 mg. of riboflavin. Group C, 10 subjects, ate the regular hospital diet. All subjects on the controlled diets received a daily supplement containing thiamine, niacinamide, pyridoxine, calcium pantothenate, biotin, folic acid, ascorbic acid and vitamins A and D.

In order to avoid the possibility of an amino acid deficiency, 10 to 15 grams of calcium caseinate were included in the daily diet. As before, food consumption was regulated with extensive precautions to eliminate possibility of error. A three month period of observations, during which all the subjects were on a diet con-

taining 1.1 mg. of riboflavin, preceded the experimental period. The purpose of this preliminary period was to observe and eliminate any subjects who might later prove undesirable for one reason or another.

The original plan called for an experimental period on the 0.55 mg. diet of at least one year's duration, however, the appearance in 3 subjects of dermal lesions necessitated supplementation in these 3 during the 9th and 10th month of the period of depletion.

The urinary excretions of the subjects in the A group can be summarized by stating that the average subject dropped from an original excretion of 0.4 mg. in 24 hours to a level of 0.112 mg. on the 1.1 mg. diet used in the preliminary period. Seven days after the 0.55 mg. diet was started the average excretion in the A group dropped to 0.05 mg. This level persisted for about 9 months and then fell to an average of 0.036 mg. per day. Excretions after supplementation with riboflavin depended upon the size of the supplement. Thus 2 mg. of riboflavin when given once, twice or three times a day, produced signs of tissue saturation as determined by the 4 hour load test at 38, 25 and 11 days, respectively. Some justification for the larger doses used clinically might be derived from these figures.

On all diets except the lowest, 0.55 mg. per day, the urinary excretion of riboflavin at 3 months remained constant for at least a year, but on the 0.55 mg. diet the average 24 hour excretion dropped from 9.3% of the ingested riboflavin to 5.3% a year later. The per cent excretions at dietary levels of 0.75, 0.85 and 1.1 mg. per day were approximately the same, i.e. about 10%. At some level between 1.1 and 1.6 mg. per day there is a marked increase to approximately 30%. This may

mean that the average basal requirement is somewhere between 1.1 and 1.6 mg, of riboflavin per day. These values are in general agreement with those reviewed by Parsons, and Brown, Porter, Ingalls and Ohlson. Emergency requirements during illness and for repair of injured tissue may be greater.

Of the large number of clinical tests made on the depleted subjects, only a few produced positive results. The investigation of the eyes was particularly comprehensive and included tests of visual acuity, flicker fusion, tear flow, dark adaptation, pupil size and contraction, corneal sensitivity, interocular pressure and frequent slit-lamp examinations for corneal vascularization. Only one of these tests gave evidence of changes associated with riboflavin deficiency, and that was a test for flicker fusion devised by Dr. Liebert.

The ability of the subjects to perceive flicker was determined by using a bulb which was illuminated and extinguished at different controlled rates. The point at which the light interruptions were just recognized was accepted as the threshold of flicker fusion. The test, when done at a distance of 6 feet from the source of flicker, showed a marked increase in the threshold of 3 subjects of the A group at about 9 months after the beginning of the 0.55 mg. diet. None of the subjects of the control group (B group) showed similar changes. No other eye changes could be correlated with riboflavin deficiency.

As expected, it was the skin which produced the first manifestation of possible deficiency of riboflavin. Four months after the beginning of the 0.55 mg. diet, one subject had a lesion at the left oral angle which was diagnosed as a typical herpes simplex vesicle. During

the succeeding 6 weeks a definite horizontal fissure with crusting appeared at both oral angles. Local therapy with zinc oxide and camphorated oil assisted healing of the right oral angle in less than 3 weeks. That on the left, however, remained in a fluctuating state of remission and exacerbation and did not heal completely until after riboflavin was administered. In addition to the angular stomatitis, this subject had a scaling, crusting lesion of the scalp which was resistant to routine therapy with sal-sulphur ointment. A similar condition was present in the hairy areas of the chest. On the face, severe vertical fissuring appearing at the naso-labial folds and eroded through the muco-cutaneous border into the nostrils. Small fissures also appeared at the nares. All of these conditions healed very rapidly after administration of riboflavin.

Two other subjects in group A developed similar lesions which were noted some months later.

Twelve out of the 15 subjects in group A showed changes in the scrotal skin which were very interesting. This was first observed in one subject in the 8th month of the 0.55 mg. diet. The anterior surface of the scrotum was markedly reddened and there was scaling and desquamation of the superficial layer of the skin. The lesion was discrete, bilateral and not connected in the mid-line. During the eighth to the tenth month scrotal lesions became apparent in other deficient subjects. It was necessary to treat the more severe of these lesions with riboflavin by mouth as soon as it became apparent that topical treatment was not successful. Recovery after the administration of riboflavin was dramatic.

After the scrotal lesions had appeared in group A, and just about the time when we thought we had a spe-

cific lesion for riboflavin deficiency, 3 subjects in the B group developed a scrotal erythema with little or no scaling. This prompted an investigation of several hundred other hospital patients, who presumably were adequately nourished. Scrotal erythema but no scaling was found in a large percentage of those who were incontinent. The basic irritation of the skin of the subjects was probably due to poor hygiene. However, no bacterial or mycotic infection could be found. Explaining the more severe lesions in the A group, we might say that, in the absence of adequate riboflavin, tissue resistance became less, repair became retarded and the development of the more severe manifestations of scrotal dermatitis was promoted. The possibility that cleaning agents used in the hospital laundry may have been a contributing factor was considered but not established.

The three subjects who had the most severe oral and scrotal lesions had auburn beards. This may or may not have been a coincidence, but it is thought provoking to add to this fact the observation that the one subject in the previous project who developed angular stomatitis is a distinct "red-head". For almost a year this man had been on a diet that provided 0.75 mg. of riboflavin per day. The significance of the rather severe left oral lesion which spread over his left cheek was minimized because of a history of minor oral lesions which developed when the subject had been receiving riboflavin as a dietary supplement. This is another demonstration that such oral lesions are not necessarily due to riboflavin deficiency, but, when they become chronic and do not heal readily until after riboflavin is provided, then ariboflavinosis is probably a proper diagnosis.

Thus, past disagreements on what is or is not riboflavin deficiency might be due to the trauma to which the body is subject, the extent and quality of which varies greatly in different environments. In evaluating these skin changes it is not the specific lesion but the retardation of healing which should be given primary consideration.

The study of lactic and pyruvic acid levels of the subjects on the diets deficient in riboflavin showed no changes in any way similar to those obtained when the diet was low in thiamine. The yellow enzymes are necessary in such a large number of cellular reactions, it is surprising that no one has found any changes in the metabolites in the body fluids which might be useful in estimating early riboflavin deficiency. It may be that the cell, once matured, requires only a minimal amount of riboflavin to keep it functioning. However, the rate of replacement of these cells with others may be dependent upon a more abundant supply of riboflavin, obtainable either from the diet or by the breakdown of less vital tissue.

One test of function which may prove useful is the test for ability to perceive flicker. This very rapid normal function may require either a greater local oxygen consumption than some slower activity, or a specific enzyme system which may be more easily fatigued in the absence of adequate riboflavin. It is hoped that we will be able to say more about this at some future date.

During the past 6 years we have been trying to obtain facts which would help establish vitamin requirements. While there is no doubt that there are certain minimum levels below which most individuals would become thiamine or riboflavin deficient, there is no such

certainty about what the optimum levels should be. We have in our laboratory, today, one rat which shows specific signs of riboflavin deficiency at levels which hardly affect his litter mates—and riboflavin alone is all that is necessary to bring his appearance and nutritional behavior back to normal. This observation might be compared with that on the 4 individuals in our projects (who happened to have auburn beards) who showed skin lesions long before any of the others on the same diet.

Individual variations were even more apparent in the thiamine project where, because of the changes in the peripheral nerves and the use of tests for accumulation of lactic and pyruvic acid, it was easier to measure pathological states. Thus 0.4 mg. thiamine per 2200 calories was obviously not adeqaute for many individuals, while others did not show any deleterious effects of this regimen.

In summarizing:

1. A three year study of 24 male subjects on diets restricted in thiamine has shown that an intake of 0.5 mg. of thiamine is below the minimum requirements of men who are relatively inactive and whose daily food consumption provides no more than 2200 calories.

2. The normal relationship between blood glucose levels and blood lactic and pyruvic acid levels was investigated and it has been demonstrated that the glucose levels must be considered when evaluating levels of lactic and pyruvic acid.

3. A metabolic load test has been devised which may be useful in the diagnoses of early or mild and cryptic states of thiamine deficiency.

4. The effects of diets restricted in riboflavin but otherwise adequate were observed in 15 male subjects. Fifteen other subjects were observed simultaneously as controls. Depletion with respect to riboflavin on a diet providing 0.55 mg. of riboflavin in 2200 calories was maintained for from 9 to 17 months, during which period angular stomatitis, seborrhoeic dermatitis, scrotal skin lesions, and diminution of ability to perceive flicker was observed.

5. Studies of excretion of riboflavin in the urine suggest that the riboflavin requirement of a resting adult is between 1.1 and 1.6 mg. per day. A reserve of riboflavin cannot be maintained on levels of intake below 1.1 mg.

6. An intake of riboflavin below 0.6 mg. per day is insufficient to promote normal tissue repair.

7. The type of abnormality encountered in riboflavin deficiency will be dependent largely upon the trauma, irritation, infection or other injuries to which the tissues are subjected. Riboflavin is necessary for tissue growth and the healing rate of injured tissues is limited when adequate riboflavin is not present.

## Discussion

Dr. ROBERT M. KARK (University of Illinois, Chicago): Most of us have read and followed with great interest Dr. Horwitt's beautiful studies.

During the past years there has been a dearth of cases of florid beriberi in Chicago for us to study. Recently, Dr. Horwitt, my other colleagues and I have had an opportunity to study the first case of beriberi heart disease we have diagnosed in three years. This

patient had zero levels of thiamine in his urine, and showed a tremendously increased carbohydrate index. This confirms Dr. Horwitts' original concept that the carbohydrate index is of use clinically.

However, one would have to see more studies of malnourished patients in the future in order to know whether his index is specific for the diagnosis of beriberi. It may be that the carbohydrate index is also disturbed in other types of malnutrition.

The second point which I should like to bring out and ask some questions about concerns peripheral polyneuritis. There is controversy about the etiology of this condition. Many clinicians, including myself, feel that our patients ill with polyneuritis do not respond to therapy with thiamine. Dr. Horwitt's patients, when he observed them, developed polyneuritis. However, he treated them not with thiamine but with a B complex mixture obtained from yeast.

Recently Dr. William Bean has shown dramatic changes in patients with polyneuritis after injection of vitamin B-12. I wonder if Dr. Horwitt has any data on the vitamin B-12 levels of his diet, and on the vitamin B-12 content of the yeast extract with which he treats his patients?

CHAIRMAN YOUMANS: It might be worth while to point out that similar discrepancies in the results of treatment were observed in prisoners of war held by the Japanese.

Dr. HORWITT: It is unfortunate that B-12 was not available at the time we did this work. We have since wondered and worried a little bit as to why we didn't

use all the pure vitamins when the thiamine project was set up. In the subsequent project, we made a more definite attempt to use the pure vitamins. There was a lot of discussion before we decided to use the yeast extract.

It is not possible for me to say definitely that B-12 was adequately considered in the first project. Thiamine, when given to one or two patients in pure form later in the work, did give us therapeutic results; but the large majority of the patients did obtain yeast extract. We were impressed at the time that the responsible factor was thiamine.

Dr. GEORGE R. COWGILL (Yale University, New Haven): Why did you use a complicated yeast extract instead of thiamine in studies where you were trying to limit your variables and were particularly interested in thiamine?

Dr. HORWITT: At the time we set up the project, thiamine was not the major interest. We were interested in the B complex. There were some long, involved discussions by a committee of the National Research Council as to what should be used. The majority opinion at the time was to use a yeast extract. In later months we were unhappy about that, but we had to go through with our first decision.

DR. OTTO A. BESSEY (University of Illinois, Chicago): In connection with the comments, may I remark that, in experimental animals, where one can control conditions quite well, there is no question but that polyneuropathy can be produced by thiamine deficiency, pure thiamine deficiency. Also one can repair

such nerve degeneration with thiamine, and thiamine alone. How rapidly that repair occurs depends upon how much damage has occurred. The nature of the lesions in polyneuropathy of the thiamine type is such that degeneration starts at the nerve ending. One is dealing with long nerve fibers, with degeneration starting at the periphery. One can follow the length of time such neuropathy has been present by histological section, and can show that there is a relationship between the extent of degeneration up the nerve fiber and the length of time required to repair that nerve to a symptom-free and to a histologically satisfactory situation. If the degeneration develops beyond a certain degree it cannot be reversed, which of course is a fundamental bit of pathology quite well known in connection with nerve degeneration. I think experimental evidence is quite clear that one can produce such neuropathies with thiamine deficiency, and one can repair them with thiamine therapy. This, of course, does not exclude the possibility of neuropathies due to other causes. In such an event one would not expect thiamine therapy to be beneficial. It certainly would be erroneous to conclude that all neuropathies are due to thiamine deficiency, and that all of it can be reversed by thiamine therapy. It would be equally erroneous to conclude that thiamine deficiency does not produce a polyneuropathy, or that such a polyneuropathy can not be reversed by thiamine, providing it hasn't gone so far as to become a pathological situation that cannot be reversed.

DR. HARRY A. WAISMAN (University of Illinois, Chicago): In answer to Dr. Kark's question, I might quote some of the work we did on monkeys with thiamine deficiency.

We used liver extract in the control diet to which crystalline vitamins were added. If the liver extract was sulfited, the thiamine was selectively destroyed and we did get a pure thiamine deficiency. The sulfited liver extract was fed at a 3 per cent level. Electrocardiographic evidence on the monkeys convinced us we were dealing with a true beriberi heart and a pure thiamine deficiency.

In support of Dr. Bessey's statement that thiamine will reverse a neuropathy specifically due to thiamine, our monkeys did recover completely. The lost weight was regained and the electrocardiographic changes were improved. All clinical signs of unsteadiness together with biochemical changes such as increased pyruvic acid or lactic acid levels were also reversed by simple additions of thiamine.

DR. L. EMMETT HOLT, JR. (New York University, College of Medicine): I might say a word about the experiments that Dr. Najjar and I did in Baltimore several years ago. We used a synthetic diet and, with the elimination of thiamine only, we produced definite neuritis in three individuals. The neuritis healed slowly. We first treated it with thiamine supplements alone, changing later to a regular diet with a yeast extract when it was perfectly clear that healing had commenced and was progressing satisfactorily.

DR. RICHARD W. VILTER (University of Cincinnati, College of Medicine): I would like to say "Amen" to Dr. Bessey's statement. Thiamine in certain circumstances will lead to complete recovery in neuritis. It depends upon the length of time and also upon what other factors are deficient in that individual. There are plenty of examples of nutritional neuritis which thiamine will heal.

# STUDIES ON B VITAMIN REQUIREMENTS OF INFANTS

L. EMMETT HOLT, JR.,
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It is not possible to ascertain the nutritional requirements of infants by extrapolation of data on adults or from animal experiments. The growing infant is a species *sui generis* and must be studied directly.

There are peculiar difficulties in studying infants on experimental diets. Ethical questions arise which make it important to define the types of study which can justifiably be undertaken. The physician responsible for the welfare of his patients cannot subject them to hazards even if the consent of responsible guardians were to be obtained. His task is to protect them. The use of experimental diets would appear to be justifiable under three circumstances: (1) when the study is without risk, (2) when the study, though perhaps involving risk is designed to benefit an ailment and (3) when the net result of the study is beneficial to the patient, regardless of any ailment he may present. In this last category may be included studies which, though involving minimal risks, protected the subjects from greater risks—infants receiving sub-optimal care for whom a nutritional study provides optimal care with an over-all reduction in hazards to health. In the studies I shall describe to you we have been guided by these principles. Although individual nutrients have been temporarily restricted or withheld, an endpoint short of clinical deficiency was employed and, although

in a few instances evidences of clinical deficiency were encountered, there is no indication that any of our subjects was harmed; on the contrary there is considerable evidence that they were individually benefited by the protection and care which the study afforded.

I shall try to cover briefly the work we have been doing on the B vitamin requirements of infants during the past four years. Some of these vitamins have been extensively studied; on others we have only fragmentary information.

*Thiamine*

Our first studies were concerned with thiamine, and illustrate the technique we have employed in ascertaining minimal requirements of the B factors in general. We have studied the urinary excretion of these vitamins on controlled intakes, using as an end point a characteristic break in the excretion curve which occurs as the intake is lowered, known as the "point of minimum excretion". As one lowers the intake of thiamine, the output of thiamine in the urine decreases in a linear fashion until this critical point is reached. Further decreases in intake cause virtually no further reduction in urinary output, even when the intake is reduced to the point of clinical deficiency. It is our concept that excretion above the minimal level represents a surplus above minimal requirements. Although the intake can be considerably reduced below the point of minimal excretion before symptoms are encountered, it is possible that subclinical changes are developing under these conditions. Our procedure therefore consisted in adjusting the thiamine intake of infants fed on a

synthetic or purified diet* to determine accurately the point of minimum excretion. The endpoint was an excretion barely above the minimum level.

I shall digress for a moment to present some evidence obtained from rat experiments bearing out the significance of the point of minimal excretion. In these studies by Salcedo, Najjar, Holt and Hutzler, tissue concentrations of thiamine at various levels of depletion were correlated with urinary excretion. In the case of tissues capable of storing thiamine (e.g., heart, voluntary muscle, liver, kidney) depletion caused a gradual reduction of thiamine content without a sharp break. In the case of the brain, however, which apparently cannot store thiamine, the concentration of this vitamin remained constant until a critical point was reached after which it fell abruptly. A close correlation was found between this critical point and the point of minimal urinary excretion.

The point of minimal excretion for infants between 10 and 15 pounds in weight was encountered at an intake approximately one-third of that which had been found by Najjar and the author to produce this phenomenon in adult subjects. The break in the urinary excretion curve occurred at intakes between 140 and 200 micrograms a day in 7 infants (Holt, Nemir, Snyderman, et al) as contrasted with 500 to 600 micrograms for adults. Our figures for the point of minimum excretion have since been extended and confirmed in further studies and it seems possible to speak with

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* The diet consisted of vitamin free casein (providing 15% of the calories), crisco (providing 35% of the calories) and vitamin-free dextrimaltose (providing 50% of the calories). A mineral mixture was added to this. Fat soluble factors were supplied by a fish oil concentrate, water soluble factors being supplied as a supplement, of which only the factor under study was varied. This supplement was given in divided doses.

a reasonable degree of assurance as to minimal thiamine requirements in infants of this weight group under the conditions of the study.

The question remained as to thiamine requirements when the conditions were varied. We first attempted to ascertain the effect of varying the proportions of protein, carbohydrate and fat on the thiamine requirement. Evidence was obtained that thiamine requirements were appreciably affected by the proportion of carbohydrate in the diet. An attempt to measure this effect quantitatively was carried out in the following manner. An infant on our standard formula (caloric distribution P.15%, F.35%, C.50%) was maintained on a fixed thiamine intake (200 micrograms a day) which permitted urinary spilling appreciably above the minimal level. The carbohydrate calories were then increased to 85 percent by isocaloric substitution of carbohydrate for fat. This was promptly followed by a fall in thiamine excretion to the minimal level. The thiamine intake was then gradually increased to 240, 260 and finally to 280 micrograms a day. Only at this last level of intake did the urinary thiamine excretion rise to the previous level. We can thus measure the thiamine required for the combustion of the additional carbohydrate calories in the infant or, conversely, the thiamine sparing action of fat.

A possibility which interested us further was whether different types of sugar would affect the thiamine requirement. Najjar and the author have shown that biosynthesis of thiamine occurs in the human, and it was conceivable that this might be of practical importance and might be affected by the particular carbohydrate fed. We therefore made exploratory studies with infants adjusted on a fixed thiamine intake pro-

viding an excretion detectably above the minimum level; when equilibrium had been attained, the sugar was varied. It was found that substitution of cane sugar, galactose or levulose for the dextrimaltose in our standard formula failed to influence the thiamine excretion. Such variations in the sugar may thus be disregarded in considering thiamine requirements.

We next undertook to study the role of bacterial synthesis of thiamine in the intestine. It was clear that such synthesis occurred, but not clear whether it occurred under conditions permitting significant absorption of thiamine to occur. Thiamine might be formed by the bacteria, but not liberated high enough in the intestine or liberated in such form as to be readily absorbable. We attempted to evaluate the contribution of the intestinal bacteria by experiments in which a shift to parenteral nutrition was made, the thiamine in both periods being given parenterally. It was found that the substitution of parenteral for oral feeding failed to reduce the thiamine excretion in the urine, thereby indicating that starving out the intestinal bacteria did not affect the thiamine economy of the body. We attempted to evaluate this factor further by giving an adsorbent (kaolin) and by an antibiotic (sulfasuxidine). When we gave kaolin there was no change in thiamine excretion. When we gave sulfasuxidine the intestinal bacteria were definitely inhibited. The stool thiamine decreased to zero, nevertheless the urinary thiamine remained unaffected. It would therefore seem quite clear that the thiamine produced by the intestinal bacteria is of little nutritional significance.

An anomalous situation appears to exist in the breast-fed infant who according to accepted analyses commonly receives less than 100 micrograms a day and

does not exhibit symptoms of thiamine deficiency. This contrasts with our estimated requirement of 140 to 200 micrograms a day. It has been supposed that the bacterial flora of the intestine on the breast milk diet favor thiamine synthesis. We attempted to study this situation by determining the urinary thiamine excretion on a breast milk diet to ascertain if on this low intake it was nevertheless above the minimal excretion value. Only preliminary observations are available at the present time on 3 infants fed on preserved breast milk obtained from the Mother's Milk Bureau in New York City. Thiamine analyses of this milk gave values comparable to those in the literature. The urinary thiamine of these infants fell to the minimal level shortly after the breast milk diet was instituted and rose above this only when a supplement was given sufficient to bring the thiamine intake up to our previously determined requirement levels. We hesitate very much to draw the conclusion that breast milk in general is dangerously low in thiamine in the face of clinical experience to the contrary. The observations require extensive confirmation with fresh breast milk and the relation between maternal thiamine intake and milk thiamine needs to be further studied, but it may well be that the breast milk of many women does not provide a desirable margin of safety.

Before concluding my remarks on thiamine, I should like to call attention to one more variable affecting the requirement, namely, the destruction of thiamine by sterilization of the milk. The thermolability of thiamine is well known. Although the destruction varies with the degree of heat applied, the duration of the heating is of even greater importance. Efforts to protect the infant from harmful bacteria have resulted in

increasingly strenuous sterilization procedures, without considering the possibilities of nutritional damage. In addition to initial sterilization at the dairy or processing plant, terminal sterilization in the home or hospital is generally practiced and terminal sterilization by autoclaving or bubbling steam has become the rule in many institutions. The destruction of thiamine by the initial processing is usually negligible, but the extent of its destruction by the terminal procedures seemed desirable to investigate. Here again we have only preliminary observations to offer, but we have observed that autoclaving at 15 pounds for half an hour will destroy enough thiamine to bring most babies fed on ordinary cow's milk formulas down to the point of minimum excretion in the urine and that less strenuous terminal sterilization will sometimes do this. It would seem that the more strenuous terminal sterilization procedures may make it desirable to fortify the milk.

*Riboflavin:*

It has already been pointed out by Dr. Horwitt that in adults the riboflavin excretion curve shows a sharp break as the intake is reduced below a critical point, further decrements causing only a slight further reduction in output as compared to the very marked change in output when intake is altered above this level. We have similar data to present in regard to infants. In 5 subjects we have determined the "point of minimal excretion"—perhaps the term "point of critical excretion" is preferable here—and we (Holt, Snyderman, Ketron, et al.) have used this as a measure of the minimum riboflavin requirement of the baby. This point has been found at an intake of 400 to 500 micrograms of riboflavin per day in infants 10 to 18 pounds in

weight. Thus it appears that the infant requires between 2 and 3 times as much riboflavin as he does thiamine. His relatively large requirement as contrasted with the adult is also worthy of comment.

We have additional information to present confirming the significance of the point of minimal riboflavin excretion in the baby. Through the courtesy of Drs. Bessey, Lowry and Burch, measurements were made of the riboflavin of the serum, of the red cells and of the white cells in two infants whose riboflavin requirement was being determined by variations in intake differing only minimally from the critical value as determined by urinary excretion. At the onset of these observations, following a period of complete riboflavin deprivation the blood values were subnormal, but during the course of many weeks of adjustment around the minimal range the blood values gradually returned to normal. Thus it appears that the minimal riboflavin intake, as determined by the urinary excretion technique is an intake which will slowly cause repletion of partially depleted cells. This observation was confirmed in two more infants whose blood riboflavins were studied by Dr. Robert Shank.

We made observations on the effect of varying the proportions of calorigenic foodstuffs on the riboflavin requirement, but were unable to demonstrate any change in requirement by the urinary excretion technique. Likewise individual carbohydrates—sucrose, glucose, lactose, galactose and dextrimaltose all gave identical results.

The contribution of the intestinal bacteria to the riboflavin economy was studied by attempting to eliminate the bacteria by parenteral feeding and by sulfa-

suxidine—all without noticeable effect on the urinary riboflavin excretion maintained just above the minimal level. Hence we feel that riboflavin synthesis by the intestinal bacteria, though it unquestionably occurs, is of little nutritional significance.

An interesting observation was made in the course of these riboflavin studies. Every now and then we would get a child who was well adjusted, excreting a constant amount of riboflavin in the urine on a fixed riboflavin intake, in whom there would suddenly occur a large outpouring of riboflavin in the urine. We were able to relate this phenomenon to the weight curve of the infant. When for any reason the infant stopped gaining weight an increased spill of riboflavin would occur in the urine. There would seem to be a practical implication here concerning riboflavin therapy. Under the influence of acute disease when tissues which store riboflavin are disintegrating there is apparently an abundance of circulating riboflavin, giving rise to the increased urinary excretion. At such times riboflavin therapy would not appear to be indicated. The need for riboflavin would seem to be during convalescence when reconstruction of the destroyed tissue is the concern of the organism.

We have carried out a few studies on the riboflavin excretion on breast milk to ascertain whether an adequate margin of safety is present for this vitamin. Again the results are preliminary and do not at the present time permit the deduction that breast milk is deficient, although the margin of safety may be considerably less than has been generally believed.

*Niacin:*

In measuring nicotinamide requirements we have made use of the excretion of N-methyl nicotinamide in the urine which is known to fall to a minimal level at inadequate intakes of the vitamin. The observation was made that infants receiving 15% of the calories as casein maintained excretions above the minimum level for N-methyl nicotinamide even when no supplement of the vitamin was given. A reduction to the minimal level could be obtained in two ways—by reducing the protein intake or by substituting a biologically poor protein for casein. It would appear that there is sufficient tryptophane in casein to meet the nicotinamide requirements when casein is fed at this level. This is in keeping with the observations of Goldberger that experimental pellagra could not be induced when milk was included in the diet.

The importance of the intestinal bacteria in providing nicotinamide was studied as had been done in the case of thiamine and riboflavin, but with slightly different findings. Some evidence was obtained of a reduction of N-methyl nicotinamide in the urine following the administration of sulfasuxidine, suggesting that biosynthesis of nicotinamide is of some value. This observation is in conflict with data reported by Najjar and myself in adults, but is in agreement with a report by Ellinger and Benesch. No explanation for the discrepancy in these results is evident.

We were interested in the site of conversion of tryptophane into nicotinamide and attempted to ascertain whether this occurred in the intestine or in the tissues by giving tryptophane orally or parenterally and measuring the resulting increased spill of N-methyl nicotinamide in the urine. Snyderman, Ketron, Carre-

tero and the author found that an equally marked and equally prompt excretion of the latter substance occurred no matter by which route the tryptophane was given. The tryptophane-nicotinamide conversion test has proved valuable in another connection, for as will appear below it serves as a useful measure of the adequacy of pyridoxine in the diet.

*Pyridoxine:*

We shall now report observations on two infants given a pyridoxine deficient diet for therapeutic purposes. They are included in the present discussion because of the unexpected nutritional implications of the observations made. These infants were mentally defective and there was reason for believing that a pyridoxine deficient diet might be beneficial. Some preliminary observations on blood pyridoxine had shown levels above normal and it was reasoned that, since pyridoxine is concerned in the conversion of tryptophane to nicotinamide this metabolic path might be occurring to the detriment of normal protein anabolism. The attempt was consequently made to carry the restriction beyond the point of urinary minimal excretion. The study failed to reveal benefit to the mental state, but proved to be of nutritional interest since clinical manifestations attributable to pyridoxine deficiency made their appearance, clearing promptly when pyridoxine was given and thus providing definite evidence that pyridoxine is a human dietary essential.

On the deficient diet urinary pyridoxine derivatives fell promptly to a minimal value and weight subsequently became stationary. In the course of weeks a hypochromic anemia developed in one infant and convulsions in the other. The convulsions were promptly

terminated by pyridoxine and normal weight gain was resumed. The child with anemia was likewise given pyridoxine which was followed by a reticulocyte response and return of the blood to normal, weight gain being promptly resumed. Of particular interest is the fact that the conversion of tryptophane to niacin disappeared early in the course of this study and returned only some time after symptoms had disappeared and gain in weight had been re-established. It would seem as if other demands for this vitamin took priority over this reaction, loss of which may thus prove to be a most sensitive test of impending pyridoxine deficiency.

*Folic Acid:*

During the first two years of our studies in infants and during 3 years' previous study on adults and adolescents in Baltimore, folic acid had not been used in our synthetic diet, indicating that, under these conditions at least, it was not a human dietary essential. A single observation in the case of an infant who had occasion to be given 3 courses of antibiotics—streptomycin and sulfasuxidine—caused us to qualify this view and indicated that when intestinal biosynthesis of folic acid is inhibited by such drugs a true folic acid deficiency can develop. Since then folic acid has been included routinely in our studies with purified diets. The manifestation of folic acid deficiency which we encountered was a macrocytic anemia associated with cessation of weight gain. Both responded promptly to folic acid therapy.

*Biotin:*

The importance of this vitamin as a human dietary essential has been questionable. Biotin was not avail-

able during the first years of our study and was hence not included in our purified diet at that time. The absence produced no recognizable ill effects. Two infants were given raw egg white as their sole source of protein for 3 months and failed to exhibit any evidence of difficulty despite low urine values of this factor. Hence there would seem little occasion for concern about the inclusion of biotin in infant diets.

*Unknown B Factors:*

When infants are fed on purified diets as described in these studies they appear to thrive quite as well as on natural diets for a period of approximately six months. In most cases, however, there has developed at about this time a failure to gain weight and in some instances a slight loss of weight without evidence of disease, the infants remaining well and happy. Restoration of normal diet at this time has caused a prompt resumption of weight gain. With the thought in mind that some essential factor might be missing from the purified diet we have attempted to supplement it—in the presence of such manifestations—in a variety of ways. Ineffective as dietary supplements were; crude casein, strepogenin (insulin by mouth), doubling the vitamin mixture, liver, biocytin and vitamin B-12. Brewer's yeast has been completely effective in some instances and partially effective in others, though often with a delay of some days or even as long as 3 weeks. The nature of the needed supplement remains unknown.

In concluding this brief report of our work, much of it obviously incomplete, I should like to acknowledge the help of a number of co-workers—Dr. Rosa Lee Nemir, Dr. Rosario Carretero, Dr. Soledad

Morales, Miss Katherine C. Ketron and, most particularly, Dr. Selma E. Snyderman.

### Discussion

DR. ANCEL KEYS (University of Minnesota, Minneapolis): I should like first to express my general admiration for this work, as well as for Dr. Horwitt's work.

There are several points which I think should be examined, particularly in regard to the riboflavin studies. You will note the change in riboflavin excretion on the occasion of weight loss. I am reminded of work, much of it unpublished, wherein we observed quite striking effects of this same nature. We observed tremendous outpourings of riboflavin in persons on constant intake and in metabolic balance under conditions of bed rest. We also observed similar phenomena under conditions of starvation.

In the child, when weight gain ceases, we presumably are removing from the body the necessity for creating new tissues and using vitamins in the structure of those tissues and in the metabolic process of growth itself. In the adult, the loss of weight may be something very different indeed, because in that case we have the possibility that there may be an actual liberation of vitamins from the contained tissues as they are metabolized for caloric purposes, and the vitamin supply is increased by that means.

Such considerations should make one think rather carefully about the whole question of the expression of vitamin requirements. Requirements for what? How can we resolve some of the conflicts, and how can we

make some sense out of the apparent differences at different ages and under different conditions?

Long ago, I hope, we gave up the idea that we can properly express requirements simply in terms of so much per person. I think the attempt for some of the vitamins to express requirements per kilogram of body weight have been disappointing, although apparently it is an improvement over the per person method.

The attempt to express the requirement per unit of metabolism has been extensively used in regard to thiamine, and, by analogy, with riboflavin, although in the case of riboflavin I think the evidence is much less satisfactory that this method represents any improvement.

There are other elements to consider. There is the possibility that the amount or mass of metabolizing tissue is the important item. That is not necessarily the same thing as the total weight—it may not even be proportional to the total weight—nor is it necessarily proportional to the basal metabolism nor to the metabolism as we observe it for the entire twenty-four hours.

Finally, in the case of childern and persons convalescing from illness, and persons gaining weight, perhaps the basic requirement is determined primarily by the extent of new tissue replacement.

One final point that I think Dr. Holt might like to comment on further with regard to breast-fed infants: I think we must be struck by the fact that apparently nature has provided for a "titration", or has arrived at the situations where the child is spontaneously "titrated" at about where Dr. Holt arrives much later in the laboratory with his synthetic diets. That leads one to suggest the possibility that the evolutionary wisdom

of nature in some of these matters is not always inferior to the wisdom of our scientific work.

DR. KARK: May I extend Dr. Keys' remarks on riboflavin.

It has been shown very clearly that, as nitrogen is laid down in patients following burns or trauma of any kind, there is a definite relationship between the amount of riboflavin which is laid down and the nitrogen retained.

In Culver's work on patients ill with cirrhosis and in our work on cirrhotics, it was shown that the excretion of riboflavin was high at times when they were in negative balance. When they were in positive balance the riboflavin in the urine was diminished.

DR. GENEVIEVE STEARNS (State University of Iowa, Iowa City): I would like to ask Dr. Holt whether the growth in length of these babies deteriorated along with the weight—whether the two paralleled, or whether growth in length was maintained for a longer or shorter period.

We have gotten very good growth in babies on synthetic diets which did not contain added B-12. The babies maintained a good growth, according to our Iowa standards, up to nine or ten months of age. B-12 is apparently not an essential up to that time, if growth in length and weight are criteria.

DR. COWGILL: May I ask how accurately you determined the amount of B-12 which they were getting?

DR. STEARNS: We did not determine it, so I don't know. It was not added specifically. The protein was from an amino acid. The carbohydrate was dextrimaltose, so there might have been some; but there was no B-12 added to those diets. The children did get orange juice and tomato juice. The amount of B-12 was probably very small, but the growth was excellent up to nine or ten months of age without added amounts.

DR. COWGILL: The point is that the child may need it, but in small amounts. All you really determined was that it was not necessary to add a certain specified amount, or a supplement. That is something different.

DR. STEARNS: That is different; but we got good growth, throughout.

DR. WAISMAN: With the rapid influx of the use of antibiotics in pediatric therapy, Dr. Holt's observations on the use of sulfasuxidine and other antibiotics in affecting what has been called bacterial synthesis, or biosynthesis, assumes greater importance. If I remember the curve correctly as shown on the riboflavin chart, there was more riboflavin excreted in the urine than was taken in at the very low levels, and yet in the stool there was the same or even a decrease in the amount of riboflavin in the stool.

I wonder whether Dr. Holt has any observations on the practical importance of these various deficiencies during antibiotic therapy?

CHAIRMAN YOUMANS: The Chair will take advantage of its position to make a few comments, too.

I would like to link together two thoughts: The first, deals with the opening statements of Dr. Holt

with regard to the need for protection of the infant during such studies. There is evidence from another source, that of the study of mass nutrition, that the first reaction of infants or children to slight diminution in one essential nutrient is to diminish their growth and protect themselves so that no real harm results. The increased output of riboflavin in the urine at certain periods, when growth might be assumed to be stopped for a time, or slowed, is further evidence of that. If not continued too long, the child does perfectly well subsequently. We do have that protection.

I should like to point out also that, in relation to the feeding of breast milk, nature appears to have set a certain level of greater growth depending on (at least as far as those children are concerned) the composition of the milk. The use of artificial mixtures does what we have often talked about clinically, that is, exposes them to the need for greater amounts of nutrients and consequently the possible development of greater and more frequent deficiencies.

DR. HOLT: Dr. Keys brings up a pertinent point which I carefully avoided because I had no ready solution for it—namely how one should express vitamin requirements—in terms of the individual, of body weight, caloric consumption, active metabolic mass or what? Without attempting a definite answer to this question I might point out that perhaps the relative requirement of the baby and of the adult will throw some light on the question. It is also possible that the same measuring stick may not be the most suitable for all the accessory factors.

The variables which affect vitamin requirements are discouraging—the presence of at least some of the

antagonists in natural foods, the role of biosynthesis and the disturbing effects of pathological processes. Fortunately, the requirements of the baby, with his relatively constant diet, constitute a less complex problem and biosynthesis is causing less confusion than seemed likely a few years ago. There is more reason today to look forward to a relatively simple solution of the requirement problem.

In regard to Dr. Stearns' remarks I may say that we do have evidence of retarded growth in length, but have not completed analysis of these data.

As regards breast milk and the all-wise provision of nature, I should like to stress the fact that breast milk is not an entity—it is an extremely variable product—the character of the fat, the concentrations of minerals and of vitamins are within limits reflections of the maternal diet. Breast milk in the Philippines is a different thing from breast milk in Australia and there are wide variations in different economic strata in every country. We should think in terms of the maintenance of proper nutritional standards in breast milk by adequate maternal diet, rather than being content to thank the creator for breast milk as it happens to be.

# PYRIDOXINE DEFICIENCY SYNDROME IN MAN PRECIPITATED BY LOW B COMPLEX DIET AND DESOXYPYRIDOXINE

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I would like to report to you this morning on a syndrome which we have observed in human subjects. Dr. John Muller and I have observed this in human subjects in Cincinnati who have been maintained on B complex-poor diets and have been treated with desoxypyridoxine.

In order to orient you a bit, I would like to go over briefly a little of what is known about pyridoxine and its metabolism.

The existence of a substance in the B complex necessary for maintaining the health of the skin of rats was observed by Paul Gyorgy in 1934. It was synthesized in 1939 by Harris and Folkers, at which time the name "pyridoxine" was given to it. Later on other substances similar to pyridoxine were found in natural sources and were determined to be pyridoxal and pyridoxamine. There exists at the present time a B-6 group of vitamins within the vitamin B complex.

As Dr. Holt already has shown you, the metabolic end product of the B-6 group, pyridoxic acid, appears in the urine and can be measured photofluorometrically either as the alpha or the beta lactone.

Numerous reports have accumulated on the effects of pyridoxine deprivation in laboratory animals; and

because this has something to do with the observations in human beings, I want to go over them very briefly.

In pyridoxine deficient or deprived young rats, a seborrheic like dermatitis, convulsive seizures, muscular weakness, lymphoid atrophy, and a decrease in circulating antibodies occur. In puppies, a hypochromic anemia with a hyperplastic bone marrow and elevated serum iron levels has been described. In swine, also, anemia has been found. Convulsive seizures also have been reported, and lesions of the central nervous system which produce neurologic manifestations very similar to the combined system disease of pernicious anemia have been observed.

In monkeys even more abnormalities have been discovered. There is always weight loss; there are scaling skin lesions and redness and soreness of the tongue and mouth; weakness, hypertrophy of the adrenal glands, lymphocytopenia and relative increase in neutrophils, and, about six months ago, an increase in the rapidity of development of arteriosclerosis was discovered in vitamin B-6 deficient monkeys.

In man there have been some reports alleging deficiency of vitamin B-6 counteracted by the administration of this material. Spies, Bean and Ashe reported that pellagrous patients previously treated with niacin, thiamine and riboflavin, but who still had weakness, insomnia, nervousness, irritability and difficulty in walking, immediately recovered when given 50 mg. of pyridoxine intravenously.

Martin and Smith reported that cheilosis, which did not respond to riboflavin, in some instances would respond to the administration of pyridoxine.

Hawkins and Barksy reported on several volunteers who were maintained on a pyridoxine deficient diet for some thirty-four days. They were not able to determine any definite clinical effect of this deprivation period on these volunteers. You heard from Dr. Holt this morning on his studies in infants.

There also have been many therapeutic trials with pyridoxine in patients who presented lesions which, to the clinicians, resembled in some way those lesions which were observed in pyridoxine deficient animals. Even though occasionally some beneficial effects have been reported—for instance, in Parkinsonism, in seborrheic dermatitis, in certain eczematoid lesions, and multitudinously in nausea and vomiting in pregnancy, post-irradiation sickness and the nausea and vomiting following administration of nitrogen mustard—still, no conclusive evidence ever has been offered that pyridoxine plays any part in these abnormalities, or that there is any regular improvement after the administration of pyridoxine.

The pathologic physiology in pyridoxine deficient animals has been investigated rather extensively. Dr. Holt has referred to some of those observations. I might mention that pyridoxine has been shown to play a part in decarboxylation processes and that it acts as a co-decarboxylase. It also acts as a transaminase. The co-enzyme derived from pyridoxine has been shown in most instances to be pyridoxal phosphate.

Pyridoxine is also necessary for the conversion of tryptophane to niacin, as Dr. Holt indicated; and animals on a pyridoxine deficient diet, who receive tryptophane, excrete abnormal end products of tryptophane metabolism, namely, xanthurenic acid and kynurenic acid in large amounts in the urine.

Pyridoxine is involved definitely in the metabolism of fat. In pyridoxine deficient animals some of the essential fatty acids relieve the need for pyridoxine and the lesions of pyridoxine deficiency can be cleared by the administration of linoleic acid, as shown some time ago by Steenbeck, Schneider and Platz.

Recently an analogue of pyridoxine, desoxypyridoxine, has become available from the Merck Laboratories. The analogue has been shown by several investigators to have antimetabolic effect in animals. Ott showed that, in chicks, it had a definite antimetabolic effect and would lead to pyridoxine deficiency. Mushett, Stebbins and Barton showed that the pathologic changes in monkeys treated with this material, desoxypyridoxine, were identical to those obtained by pyridoxine deprivation by dietary means.

Because it had been shown by Stoerk that desoxypyridoxine inhibits the growth of lymphosarcoma implants in laboratory animals, Gellhorn and Jones attempted to treat patients with far advanced lymphomata of various types with pyridoxine deficient diets and with desoxypyridoxine over a twelve to fourteen day period. All they obtained from that experimental regime was the production of convulsive seizures in several patients. Hoster and Zanes also have reported similar results in Hodgkin's disease, that is, convulsive seizures but no effect on the lymphoma and no other clinical manifestations which might be ascribed to pyridoxine deficiency.

We were interested in desoxypyridoxine for much the same reason; but not having available the data which I have just mentioned, we went through a number of toxicity tests with the material, to be certain we

were not going to affect adversely the patients upon whom these tests were to be made.

First, patients in the hospital, on a regular hospital diet, were treated with desoxypyridoxine, starting with 1 mg. and increasing it to 50 mg., with no toxic manifestations. Following this, patients on a B complex deficient diet were treated in the same fashion. We finally got up to 200 mg. intramuscularly per day without toxic manifestations.

At this point eight patients were selected for study. They had been hospitalized because of the following conditions: Two of them had rheumatoid arthritis, which was quite inactive; two had postencephalitic Parkinsonism; one had multiple sclerosis; one had a fractured leg; one had tabes dorsalis, and one had hypertrophic arthritis. Their ages ranged from forty years to approximately seventy-five. All were treated in essentially the same fashion. They were placed on a B complex-poor diet providing 3,127 calories, 41 grams of protein and 27 grams of fat, approximately (and these are estimates) 0.42 mg. of thiamine, 4.2 mg. of niacin, 0.6 mg. riboflavin, and 0.5 mg. pyridoxine, again a very, very inaccurate estimate because not too much is known of the pyridoxine content of various foods. They were maintained on this diet for the period of the experiment, which ranged from three weeks to approximately three months. Desoxypyridoxine in varying doses, from 60 to 200 mg. a day, was given during the entire period.

As clinical manifestations appeared, observations were made at frequent intervals. Treatment was begun with thiamine, niacin and riboflavin intramuscularly. Then, after four to six days, when no change in the clinical manifestations were observed, treatment with

50 mg. or 100 mg. of pyridoxine per day, depending upon the severity of the clinical manifestations, was started.

One patient was not treated with any of the B complex vitamins before pyridoxine was added. That was done principally to show that the results obtained with pyridoxine in the first procedure were not influenced by the prior administration of the other B complex vitamins. One patient received succinylsulfathiazole for the entire period of the experiment.

In six of the eight patients skin lesions were observed. The lesions usually were observed first around the eyebrows and the lateral canthi of the eyes, later along the nose and around the nasal labial folds, and still later around the lips and chin; gradually they spread over the entire face and over the forehead. The lesion was scaly and oily and looked very much like seborrheic dermatitis. It was quite red.

Four of the eight patients developed glossitis with reddened papillae along the tip and lateral aspects of the tongue, flattening of the filiform papillae and engorgement of the fungiform papillae, with redness and soreness and increased salivation. I could not distinguish this from acute niacin deficiency glossitis.

Three patients also developed erosions of the lips and cheilosis of the angles of the mouth.

Finally, one patient, the most severely afflicted, developed an erythema of the skin of the arms and legs. On this erythematous background one could see small papules which itched considerably and which caused the patient a good deal of distress. This was the only patient who developed severe systemic manifestations.

She developed nausea, vomiting, weakness and dizziness to the point where she was unable to get out of bed and was unable to retain any food.

Lesions appeared, in one instance, in as short a time as ten days. The usual length of time it took for the lesion to appear was nineteen to twenty-one days, and in one patient two months on the deficiency regime was required.

There was only slight subjective improvement when the patients received the other major vitamins of the B complex, namely, niacin, thiamine and riboflavin. The patients in some instances said that the tongue burned less, but we could not observe any clinical change that we were willing to say was real.

After the administration of pyridoxine—the diet being continued and the desoxypyridoxine being continued—the lesions began to disappear. The quickest disappearance of a lesion was twenty-four hours, the longest ninety-six hours. The patient who was most severely affected, and who developed the systemic manifestations, took ninety-six hours to show complete clearing of all lesions. Incidentally, the systemic manifestations appeared while she was receiving the niacin, thiamine and riboflavin. The tongue usually cleared within forty-eight hours, the cheilosis healed within thirty-six to forty-eight hours, and the skin lesions showed definite change, decrease in redness, within twenty-four hours and complete clearing usually within forty-eight to seventy-two hours.

Complete hematologic studies were carried out on all of our patients. In none of them did we find any significant anemia. There was no statistically significant change in the erythrocyte counts nor in the hemo-

globin. The only change that we did find was in the patient who was receiving succinylsulfathiazole; that patient developed, over a period of two months, a macrocytic anemia that responded to folic acid and did not require the administration of pyridoxine.

The only change that was noted was in the lymphocytes. In seven of the eight patients there was a definite lymphocytopenia as measured in absolute numbers of lymphocytes. There was also, possibly, in some instances, a slight increase in neutrophils, but that did not appear to be at all statistically significant.

In two of the patients there was eosinophilia of 5 and 14 per cent respectively, which developed while the skin lesions were developing and disappeared as the skin lesions disappeared.

Various vitamins of the B complex were measured in the urine during the experimental procedure. The niacin, riboflavin and thiamine levels were at the low normal range, as we find them in our laboratories. The pyridoxic acid levels were usually within the normal range, although for the first two to five days of the experimental period, while the patients were receiving desoxypyridoxine, there seemed to be some increase in the output of pyridoxic acid. We were able to show quite conclusively that the desoxypyridoxine, an antimetabolite, did not of itself influence this test.

Xanthurenic acid outputs also were measured in the urine of these patients. Usually they ran about 25 mg. of xanthurenic acid in the urine per day while on the desoxypyridoxine and the B complex deficient diet. Tryptophane, in doses of 10 grams orally, increased xanthurenic acid output to 200 mg. Another dose of tryptophane will increase the xanthurenic acid in the same way, and then, when pyridoxine is added, the

same dose of tryptophane no longer will elevate the urine xanthurenic acid. This, I think, is our best chemical proof that we actually have induced a state of pyridoxine deficiency.

I am sorry I cannot answer Dr. Holt's question about the N-methyl-nicotinamides. Those figures are in the process of being analyzed at the present time, and I don't know myself what the answer is.

Because of the observation that monkeys develop arteriosclerotic lesions on pyridoxine deprivation, we measured blood cholesterols in some of our patients but found them to be entirely within the normal range.

A few additional observations, which may or may not mean anything—and we haven't had a chance to determine what their significance is, or if they have any significance at all: The two patients with rheumatoid arthritis who were on this experimental regime developed the most severe lesions, and their rheumatoid arthritis became worse. We also observed that young patients developed the lesions much more rapidly than older patients. Our seventy-five-year-old man developed no lesions whatsoever in a period of two months. The best nourished patients developed the lesions only after a long period of time on this regime, if at all.

For instance, previously well nourished patients upon whom we have tried this recently, didn't get any lesions at all, although the xanthurenic acid in the urine was elevated after tryptophane administration. Patients with Parkinsonism were not definitely made worse, although one individual who had Parkinsonism seemed to be getting worse and developed such a severe propulsion gait that he actually went through a window. However, before any change was made in his

regime, he improved and we were unable to say that the deprivation period actually made his Parkinsonism any worse.

Several patients have been studied who had chronic lymphatic leukemia. In only one of these was there a suggestive fall in lymphocytes from 100,000 to 40,000, but after pyridoxine was added to the regime no significant increase in lymphocytes occurred; so I don't know exactly what the meaning of that is, if any.

A number of patients with naturally occurring seborrheic dermatitis have been treated in the dermatology clinic with 200 mg. of pyridoxine per day by mouth, with no observable effect on the seborrheic lesions.

I would like to read briefly one protocol on the patient who developed the most severe lesions. "A 37-year-old unmarried colored girl, with a primary diagnosis of inactive rheumatoid arthritis was placed on the B complex-poor diet on May 12, 1949. Two days later and each day thereafter 100 mg. of desoxypyridoxine was administered intramuscularly.

"On May 24, 1949 she began to complain of pruritis of the arms and legs. Examination revealed a discrete papillary eruption on the extensor surfaces of both forearms and the anterior surfaces of both legs. A few small, reddened papillae were present on the lateral margins of the tongue.

"On May 27, 1949 she had developed lesions about the angles of the nose, the eyes and the mouth, which were characterized by excoriation, redness, desquamation of superficial epithelium, and oiliness. By the next day most of the face was involved by this seborrheic-like process.

"On May 28, 1949 she was started on a vitamin mixture given parenterally each day. "There was no improvement in the skin or tongue lesions.

"On the evening of May 30, 1949 she noted the onset of rather severe nausea and vomiting, dizziness, weakness, and a feeling of impending disaster." At that point we felt the impending disaster also, because she had been awake all night and refused food the next morning. "The skin lesions were even more extensive on the face, and a diffuse erythema surrounded the papillary lesions on the extremities. The tongue was swollen and reddened.

"Pridoxine was given immediately, in doses of 100 mg. intramuscularly, twice a day. The remainder of the regime, including desoxypyridoxine and the diet, was unaltered. In six hours the patient noted improvement in her feeling of well being. By the next morning all the nausea and vomiting had disappeared, and she felt stronger. The skin lesions were unchanged.

"Within forty-eight hours the patient had no subjective complaints whatsoever, and the skin lesions were regressing. The tongue had become normal. By ninety-six hours the face was entirely free of all lesions except for some residual pigmentation. The extremities were clear, and the tongue was normal."

I think that fairly well summarizes the work that I have to report. We believe that these lesions constitute at least part of the human syndrome of acute pyridoxine deficiency, and will provide a further means of investigation of the effect of pyridoxine on the overall metabolism of the vitamins of the B complex, of protein, of amino acids, of fat and fatty acids, and, last but not least, of the activity of the adrenal cortex.

## Discussion

DR. JAMES M. STRANG (University of Pittsburgh, School of Medicine): This particular subject is concerned primarily with the action of vitamins, but I should like to call attention to the diet that was printed on the board.

I may have misread it, but in my experience it is a most extraordinary diet. I picked up from the chart about 40 grams of protein and about 50 grams of fat, and total calories of about 3,000. That would be an extraordinary combination.

I am wondering whether Dr. Vilter will turn that into food for us. I think it is one thing to write a diet in theory, and it is quite another thing to turn it into spinach, bread and butter, and things like that which someone has to eat.

Another thing I would like to know, if I am not out of order, is how Dr. Vilter gets patients who are sick to eat 3,000 calories a day. I assume the diet as printed represents what the patient ate, and not what was ordered. My experience has been quite different. I can't get a sick patient to eat 3,000 calories a day.

DR. HOLT: May I ask Dr. Vilter one question: Do you have any possible explanation for the discrepancy between the symptoms which you observed and those which we observed?

DR. THEODORE E. FRIEDEMANN (Northwestern University, Medical School, Chicago): I am interested in your observations of lesions on the face, neck, forearms and lower extremities of your experimental subjects. Was the dermatitis observed on the trunk?

The affected areas are exposed to light and continual changes in external temperatures which undoubtedly affect the capillary blood supply and result in greater changes in skin metabolism than in the unexposed areas. To which of these factors, light or changes in temperatures or other, do you ascribe the appearance of the lesions?

DR. COWGILL: I would like to mention that some time ago, when Supplee did his work on rats, he published some colored plates. One of the pictures in that series was [that] of a lesion at the tip of the tongue. Dr. Vilter also mentioned a lesion on the tongue.

I wonder, also, whether we have here a partial answer to the question concerning diet, in that, as I understand it, Dr. Vilter was trying to provide a ration which would be low in B-6, he fed this other material to see what would happen. It is one thing to have a particular experimental objective and set up your conditions so that you can study the problem, and another to argue that that may not be important because we can't get that kind of combination of foods in some ordinary, simple diet.

DR. VILTER: As far as transposing our diet into food is concerned, there is one glass of milk a day, no more than one egg a day (usually one egg every other day), no meat whatsoever, usually pureed vegetables, the choice depending considerably upon their content of various substances. The calories are increased by intermediate feedings of crackers and jelly and by giving synthetic fruit juices between meals. That is the kind of thing that increases the calories and decreases the vitamin content.

Eventually the patients get to the point where they can't look a cracker in the face. We have used that same diet for a number of years, and have never observed any similar lesions; as a matter of fact, if one puts a pellagrin on a diet like this, 50 per cent of the time the pellagra will improve with the patient at bed rest; so it is not a diet which of itself will produce skin lesions, but it does decrease the intake of pyridoxine, a substance which we cannot estimate too accurately anyway. Just by decreasing all B complex intake we hope to decrease the intake of pyridoxine.

Answering Dr. Holt's question about the anemia and the convulsions which he observed in his infants, as opposed to the lesions which we have observed in adults, I rather think it is a question of degree and a question of the age of the subject. Almost all young laboratory animals that have been placed on pyridoxine deprivation regimes have developed anemia and convulsions, but we have never observed it in adults, nor have we been able to find any evidence in adults eating regularly a very deficient diet that pyridoxine deficiency has anything to do with blood formation. I rather imagine the situation is entirely different in a rapidly growing child or infant, and that manifestations can occur there which might not be found in the adult.

Furthermore, Dr. Holt's observations were carried out over a long period of time, and ours were short, acute experiments, which also makes a difference.

Concerning the question about dermatitis on the trunk, I can say there was no dermatitis on the trunk. It was limited to the arms and legs. I really don't know why; neither do I know why dermatitis occurs

in particular parts of the skin in preference to others in completely unrelated conditions.

DR. KEYS: May I ask one further question brought up by Dr. Vilter's reaffirmation of this dietary question: As an example, the woman who had the most severe reaction: As I understand it, she was a thirty-seven year old woman with arthritis, not very active, and not a large and obese person. On 3,100 calories a day how many kilograms a week did she gain?

DR. VILTER: I can't tell you, Doctor. Actually, these patients will eat the diet for three or four weeks and then they rebel. They just can't take it any longer. I don't know how much weight gain or loss occurred during that period of time. They will eat the diet, all of it, for about that period of time, but then it goes out the window.

# MATERNAL NUTRITION, AN ENVIRONMENTAL FACTOR OF IMPORTANCE TO THE FETUS AND MOTHER AS SHOWN BY DIETARY HISTORIES

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It has become apparent from studies of human beings, as well as animals, that environmental factors operating through the mother may affect the course of embryonic development and fetal growth and in this way, modify the outcome of genetic potentialities. Nutrition is undoubtedly one of the environmental factors that deserves much more attention in our prenatal care programs than it usually receives. Several human prenatal nutrition studies reported in this country, England and Canada, in the last ten to fifteen years, have focused our attention increasingly on important public health aspects of including evaluation of nutritional status and dietary advice in prenatal care, from early in pregnancy.

As a background to the presentation of some of the recent research findings, I shall review briefly the following considerations:

1. Woman's normal physiologic processes are greatly altered during pregnancy.

    a. Her nutritional requirements are considerably increased from the 4th month of pregnancy to term. At no time during pregnancy is the caloric requirement more than approximately 20%

above normal needs while the requirements for protein, minerals and vitamins are elevated much more; in the case of some of these, the requirement may be elevated as much as 50 to 100% above the normal requirement.

b. Digestion and absorption are often interfered with, especially in the early months of pregnancy. The period is then definitely one of stress. It is the period of most rapid growth and development in life. In the early months, the embryo is developing rapidly and, in the last two trimesters of pregnancy, the fetus is growing rapidly. The fetus makes its greatest growth in length in the second trimester of pregnancy and, in the third trimester, its greatest growth in weight.

Three types of studies have contributed to our present body of evidence that faulty nutrition during pregnancy may affect the pregnant woman or her fetus in ways not usually considered to be signs of malnutrition, namely:

(1) Animal Experiments

(2) Human Research

(3) Studies of certain natural situations where changes in the nutrition of the pregnant woman appear to be correlated with mortality and morbidity statistics.

There are, in the literature dealing with animal experimentation, many illustrations of fetal damage resulting from maternal dietary deficiency. A classical study of this type is the work done by Hart, McCollum and others at the University of Wisconsin Agricultural

Experiment Station about 1917 showing the effect of different rations upon heifers during growth and reproduction. Three different rations were used. Animals fed the corn plant ration were the healthiest and gave birth to full-term vigorous young who developed normally. The young of the animals fed the wheat plant ration were either born prematurely and were small, or were stillborn, or died within a few hours of birth. The animals fed the oat plant ration or a mixture of these rations were between the other two groups in health and their young were either weak or stillborn. Another interesting finding was that these same animals continued on the same rations repeated the following year in all essential details their reproductive records of the previous year. The results on the different rations have been said to be due to differences in one or more of the essential nutrients such as calcium, phosphorus, vitamin A, riboflavin or certain essential amino acids.

The recent work of Warkany and his associates on rats in relation to congenital malformations is extremely important. He stated that: "Genetic, infectious and actinic factors have been proved to be etiologic principles leading to malformations in mammals including man. It has been suspected many times that malnutrition of the embryo can also be an etiologic factor; . . ." Warkany has shown that in the maternal diet of the rat the presence or absence of sufficient riboflavin between the thirteenth and fifteenth days of gestation is a decisive factor in the normal development of the skeleton of the embryo. Riboflavin is known to be essential for normal growth in that it is essential to cell respiration, and it would appear to be necessary also for normal embryonic differentiation.

When the maternal diet of the experimental animal is made deficient in vitamin D, Warkany has shown that malformations of an entirely different type result. More recently these same workers have demonstrated the development of certain congenital defects of the eye together with certain other tissue defects in rats when the maternal diet is deficient in vitamin A.

Although no one thus far has proved a relation between congenital malformations in man and maternal nutritional deficiency, evidence of damage to the human fetus from various maternal nutritional deficiencies can be found in the literature. Fetal rickets, fetal beriberi, fetal scurvy may be mentioned as a few of those cited in our own and foreign literature. A severe macrocytic anemia of pregnancy is common in India and is described in the Indian Medical Literature; the maternal mortality and infant mortality are both high. This has been related to the nutrition of the mother.

What of the human studies of the last ten years and related evidence from the experiences of natural groups? Several studies have been carried out on relatively large groups of women that have included evaluation of their diets during pregnancy or have included supplements to their customary diets. Many of these have shown a relationship between the maternal diet on the one hand and the course or outcome of pregnancy and the growth and development of the fetus on the other. While these relationships are surprisingly significant in certain respects, and there seems little doubt that nutrition is an important factor, it should be made clear before discussing the findings with you that the nature of these relationships is not understood and that much more research is necessary to clarify the exact ways in which nutrition functions in these relationships.

I would like to mention briefly the work of Ebbs, Tisdall and Scott in Toronto. You will remember that three groups of women were studied (1) women on poor diets from a poor economic level whose diets were supplemented to an optimal level with food, (2) women on the same poor diets from the same economic level who served as controls, (3) a group of women from a higher economic level who were taught how to provide an excellent diet for themselves. The incidence of abortions, premature births, stillbirths and neonatal deaths was considerably higher in the control group, the supplemented diet group had the best results and the educated group showed marked improvement over those on poor diets. Not only did these two groups have healthier babies, but the women were better obstetrical risks and there were other differences in favor of these two groups.

Two English studies, worthy of mention, are a study of about 5,000 women by the People's League of Health which showed that the incidence of toxemia was 30 per cent lower in the supplemented diet group than among the controls. The incidence of prematurity, on the basis of weeks of pregnancy, was, as might be expected, decidedly reduced in this group. The National Birthday Trust Fund carried out a study on some 20,000 pregnant women, 8,000 of whom served as controls. The rest were divided into two groups who were given vitamin or vitamin and mineral supplements. All three groups received small amounts of additional milk. Statistically significant reductions in the stillbirth rate and in the neonatal mortality rate were observed in the supplemented diet groups, a slight but not significant reduction in toxemia took place. The number of maternal deaths in the whole study was very small.

We might now turn to our own studies carried out by the Department of Maternal and Child Health of the Harvard School of Public Health in conjunction with the Boston Lying-in Hospital. The earlier papers deal with 216 women and their infants and a recent report is concerned with 68 younger siblings born to 57 of these women. This group includes 57 second, 10 third siblings and one 4th sibling. The dietary histories of the 57 women going through one or more additional pregnancies were collected, calculated and evaluated in a manner similar to those in the original series. I would like to emphasize to you that the dietary histories give a picture of the *average* daily food intake for the latter part of pregnancy. The ranges within the rating scale used absorb the majority of the inaccuracies which may remain in the dietary history after our method of carefully cross-checking all data has reduced such errors to a minimum. While we recognize that the dietary history at best is limited in accuracy, we believe it to be a valuable tool in research. For this reason, I would like to call your attention to the way it is used in these studies.

The nutrients and the diet as a whole are rated in 5 categories. The mean general dietary ratings used in this way merely place the women in relative relationships to each other on the basis of their average dietary intakes, just as the pediatric ratings describing the infant's condition at birth place him relative to the other infants in terms of his physical condition. If you consider the rating scale used for the diets too high, a lower scale would not change the relative position of the women on the basis of their average dietary intakes. The findings would be the same.

When the 68 siblings were added to the original 216 cases and the results graphed, results closely com-

parable to the original series were obtained. In the 284 cases: Where the diet was "good or excellent", 95% of the infants were in good or excellent physical condition at birth; in contrast, when the maternal diet was "poor to very poor", 65% of the infants were in the poorest physical condition at birth (this means that the infants were stillborn, or died in the neonatal period, were premature or functionally immature or had a marked congenital defect); 27% were in a fair condition at birth and only 8% were in good or excellent physical condition. There is a marked difference in the average birth weights and birth lengths of infants in the three dietary categories.

Recently, we divided our original "fair" diet group into two groups, a somewhat narrower fair diet group (ratings from 2.7 to 1.8 inclusive) and a "poor" diet group of those whose dietary ratings were from 1.8 to 1.1 inclusive. The lowest dietary category, the same as before (under 1.1), is representative of very deficient dietary intake even in the minds of the most conservative. This division was made because work done since the original publications led us to believe that the relation between the condition of the fetus and a poor nutritional environment in the mother changes most abruptly as the diet becomes very poor. The division into 4 dietary categories showed more clearly the *consistent* trend toward lower physical ratings of infants with lower maternal dietary ratings and the abrupt change to a large percentage of infants in the "poorest" physical condition as the diet changes to "very poor". The average birth weights and birth lengths of each group of infants also shift with each change of prenatal dietary rating.

No information was collected about the diets normally consumed by these women before or between

pregnancies. It is my impression that, since no appreciable effort was made to change their food habits during pregnancy, the diets during pregnancy in the majority of cases represent the woman's long-time food habits. Hence, any association found between prenatal dietary rating and condition of infant at birth may be due directly or indirectly to the diet during pregnancy or to long-time food habits or to some combination of the two. It is entirely possible that nutrition during the latter part of pregnancy is a factor in certain relationships shown to exist, while long-established food habits may be important to both mother and fetus in other ways.

The data on the 68 siblings alone, arranged in the manner described for the original 216 cases, gave similar results, except that the "poor" diet group contained a considerably higher percentage of "poorest" infants than was found in the original cases. The trends in average birth weight and birth length was again consistent with each shift in the prenatal dietary rating. The constancy of this relationship in every grouping is impressive. It should be pointed out that this group of infants is a racially controlled group (over 90% are of northern European stock) and that the birth lengths are done by "pressing the baby out flat" (as one of our pediatricians was heard to describe it) and holding him on a special measuring board.

We also studied 53 matched pairs of first and second siblings. They offered a situation in which hereditary and environmental variables were in part controlled. This small human study is in many ways comparable to the animal reproduction experiments of Hart, McCollum and others.

Even with this group, the consistent shifts in physical condition of the infant, in birth weight and birth length, with each change in maternal dietary rating are striking. Thirty of the 53 mothers had the same dietary ratings during both pregnancies; 6 improved their dietary ratings by one category and 7 by two categories, while 7 mothers had dietary ratings which were poorer by one category and 5 were worse by two categories. The group as a whole stayed at about the same dietary level during the two pregnancies.

Greater motivation and more education are required to change an individual's food habits markedly than were given this group who were only seen one or more times each trimester, mainly to obtain research histories, with the nutritionist answering their questions at the end of the interview or merely making a statement to them about changes in diet such as: "It would be better if you drank more milk or had more or less of this or that food".

Improvement of the infant's condition with improvement of the maternal diet and deterioration with worsening of the maternal diet was noted, but changes were not sufficiently striking to be statistically significant. The changes in birth weight and birth length are statistically significant. Unfortunately for this study, the diets of the women stayed the same or changed only slightly during their successive pregnancies. Nevertheless, this particular series of matched siblings is unique since each mother is used as her own control, thus eliminating some of the extraneous factors that influence the condition of the babies at birth.

Before summarizing the present extent of our knowledge, we must consider England's war-time ex-

perience in respect to the pregnant woman. As a result of rationing, the diets of the poorer classes improved considerably and, for the first time in the history of England, special rations including a pint of milk daily were provided for all pregnant women and, at the same time, a widespread educational program on nutrition was instituted. Subsequently, there was a sharp drop in the stillbirth rate, the greatest drop occurring in the poorest economic districts—in Wales it fell 35%. The neonatal death rate declined similarly but to a lesser degree. All other conditions in life were said to have worsened so this improvement was largely ascribed to improvement in the nutrition of the pregnant woman.

The late Dr. Toverud's work in Oslo is also worthy of attention. Under her supervision, 1,000 pregnant women in a special health unit were given nutritional guidance as a part of prenatal care. Her results show, as do ours, a clear relationship between a good maternal diet and the course of pregnancy, as well as benefits to the infant. The stillbirth rate for the years 1939-1944 in the supervised group averaged only 16 per 1,000 live births, compared to 30 per 1,000 live births for the city of Oslo. The neonatal death rate for the same years was 11 per 1,000 in the supervised group and 20 per 1,000 live births for the city of Oslo.

While we still need much more research to add to our very scanty knowledge of how nutrition operates through the mother during pregnancy, it looks very much as though the fetus may suffer far more quickly and with less maternal disturbance than is generally appreciated, when the mother is malnourished. Since we know very little about what the effects of any given deficiency may be, it would seem wise to attempt to evaluate the mother's nutritional state as early in preg-

nancy as possible and to correct all possible deficiencies as quickly as possible. It is also important to attempt by educational means to make the woman of childbearing age realize that it is only sound common sense to enter pregnancy in good nutritional status.

## Discussion

DR. GEORGE E. WAKERLIN (University of Illinois, Chicago): I think that in the Toronto study a good correlation was found between the dietary habits of these women and their economic levels. I wonder if you have found any exceptions? Also, was there good correlation between the dietary habits and the educational levels of these women, and did you find any notable exceptions?

MRS. BURKE: The women in the higher economic group in the Toronto Study were taught to eat a better diet, and apparently did. Our own study represents largely a middle economic group.

Since this study, we have investigated some 500 additional cases which we are now putting together. We attempted to go into Richardson House, the private wing of the Boston Lying-In Hospital, so that the new study would encompass a broader economic group. In our experience, lack of education as to food values means that many diets at all economic levels may be poor. It has been my experience with children and with women that the best diets are found most often in middle economic brackets. Very wealthy women often eat poor diets, for a very different reason from that applying when you find poor diets in the lowest economic brackets, wherein money as well as education plays a part.

DR. STEARNS: I would like to ask Dr. Burke if she found that the production of twins was enough of an added drain on the mother to throw the babies into another group level than would have been true otherwise.

MRS. BURKE: I am very glad to have that question asked, because I wouldn't introduce the subject myself; but since you have brought it up, perhaps I should tell you that in the first study we had twelve sets of twins. The incidence of prematurity, the incidence of congenital malformations, and the number of stillbirths, and so on, are surprisingly higher percentage-wise in that group. We have never put out anything about the twins. There are so few cases. After we have finished some of this newer work we will probably add them to what we started to do at the outset of the new study, which is now in its fifth year.

At the beginning of the new study, where we are studying all stillbirths and neonatal deaths and all prematures under 4 lbs. and all malformed infants born in the main part of the Boston Lying-In Hospital, it was my desire to study all the twins born in the Lying-In Hospital in this five-year period. We attempted such a study in the first year, but we did not have either money or staff to continue it, as the Boston Lying-In Hospital averages about eighty sets of twins a year. I think a great deal could be learned by such a study with a large series of twins.

CHAIRMAN YOUMANS: This will conclude our morning program.

## SATURDAY AFTERNOON SESSION

November 19, 1949

The meeting reconvened at 2:00 p. m., Dr. Otto A. Bessey, Chairman, presiding.

## THE NEWER HEMATOPOIETIC VITAMINS

WILLIAM J. DARBY

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I would like to take you through some of the effects of the newer hematopoietic vitamins, the group known as the folic acids and, I think we are safe in saying now, the group known as the B-12s. The folic acid family, as you know, is chemically the pteroylglutamates.

There are at least three pteroylglutamates that occur in nature. All of them have the same nucleus, but varying numbers of glutamic acid residues. One of them, pteroylglutamic acid itself, the common synthetic folic acid, has only one glutamic acid residue. Another has three, the so-called fermentation factor or the triglutamate, and finally the conjugate, which has seven glutamic acid residues and which is present in yeast.

Chemically, these substances are quite different from vitamin B-12. The structure of vitamin B-12 has not yet been revealed, if it is known. It is a higher molecular weight compound than pteroylglutamic acid. It contains cobalt as an integral portion of its molecule, and a benzimidazole nucleus has been isolated as a degradation product.

I emphasize this because some, particularly those who have not followed the field too closely, seem to think that there is a chemical similarity between the two groups of vitamins.

These vitamins can be determined quantitatively. In most cases, however, the quantitative measurements must be made employing microbiologic techniques. There are two frequently utilized test organisms for the pteroylglutamates, L. casei and Streptococcus fecalis. The first appears to respond to the conjugated form as well as to the unconjugated form, while the other responds only to the unconjugated form. This serves as a means of differentiating the conjugated from the unconjugated forms of the vitamin. There are some chemical procedures, but their publication has been of too recent date for them to have been applied clinically and results published.

The microbiologic assays (and there are a number in the literature) for B-12 are, in general, rather unsatisfactory and do not lend themselves readily, at least, to clinical studies. When I say "unsatisfactory" I mean unsatisfactory except for attacking certain specific problems.

Both of these families of vitamins have hematopoietic activity in man. They appear to be active in a group of anemias characterized by megaloblastic hyperplasia of the bone marrow. These anemias, with megaloblastic hyperplasia, very frequently—in fact, almost invariably, if the anemia is severe enough—are associated with macrocytosis. In addition, these anemias are very frequently associated with gastrointestinal disturbances, diarrhea, glossitis, and so on.

The groups of anemias in which these vitamins act include pernicious anemia, sprue, so-called nutritional

macrocytic anemia, megaloblastic anemia of infants, and pernicious anemia of pregnancy, or so-called pernicious anemia of pregnancy.

I should like to consider with you the role of these factors in each of the five anemias that I have mentioned. First let us think about pernicious anemia. Either folic acid or vitamin B-12 will institute a hematopoietic response in most cases of pernicious anemia.

There have been a number of efforts to compare the activity of folic acid to liver extract, what we suspect is the same as comparing it to vitamin B-12 since liver extract contains vitamin B-12. I think it is fair to say that the initial hematopoietic activity (and I confine myself to that point at this moment) of folic acid in pernicious anemia is almost identical with that of liver extract. However, the response may be somewhat slower. Instead of reaching a maximum in about two and a half months, it may take as long as four months for maximum blood levels to be attained. However, usually the patient will attain maximum erythrocyte levels following administration of folic acid, in our experience.

Once the maximum level is obtained and the patient's therapy is continued, any one of five different things may happen to a patient with pernicious anemia treated with folic acid.

Following the attainment of maximum blood levels, the patient may then gradually, despite continued therapy, relapse hematologically. This may be a simple hematologic relapse. With an increase in the dose, no increase in reticulocytes and no increase in erythropoiesis occurs.

The patient may have very little, if any, hematologic relapse, but may suddenly develop a neurologic relapse.

A third type of response is that the occasional patient may be maintained hemotologically but may develop a distinct glossitis, which later will respond to liver extract or vitamin B-12.

Another response that we have observed is that, in the rare case, a patient will go for at least as long as three years receiving folic acid alone, with satisfactory maintenance. This does not mean that we recommend folic acid alone as treatment for pernicious anemia. It is an interesting observation. Its significance I cannot explain.

Finally, there is a small group of patients with pernicious anemia who will not respond initially to folic acid. There have been three or four such cases that have come either to our attention or to the attention of some of the workers in the area.

It is apparent, then, that folic acid is not complete replacement in pernicious anemia. As far as I am aware, vitamin B-12 has been demonstrated to the present date as being complete replacement in cases of pernicious anemia.

Let us turn our attention for a moment to another of these anemias, sprue.

Satisfactory hematopoietic maintenance apparently can be obtained in patients with sprue treated with folic acid alone. At the moment I can think of one patient who has been going for approximately four years on folic acid alone, and has been maintaining a red count of around 5,500,000.

All cases of sprue, however, will not maintain their red cell levels on treatment with folic acid alone. It appears that some cases of sprue, despite initial satisfactory hematologic responses to folic acid, later either develop an additional need for vitamin B-12 or else become depleted of vitamin B-12. Possibly because they are on a deficient diet or they don't absorb quite as well as other people, the first deficiency which becomes manifest is that of folic acid. This type of finding leads me to suggest that some patients are going to need both factors in order to meet completely their dietary requirements.

So far we have talked only about the hematopoietic response in sprue. Now let us consider the gastrointestinal defects.

You will recall that in sprue there is impaired gastrointestinal absorption, decreased absorption of fats in particular. There is a flat vitamin A tolerance curve. Vitamin K deficiency develops. Another evidence of poor absorption is a flat glucose tolerance curve. Patients treated with folic acid, when they are in the reversible stage of sprue, will show alterations in all of these findings.

The glossitis of sprue responds to folic acid. We have seen no cases of sprue in which glossitis has recurred while the patients were getting folic acid; even those patients who had hematologic relapses did not have a return of glossitis, despite the fact that they had had glossitis when they were first treated with folic acid. What this means, I do not know.

In sprue we are not concerned with the neurologic complications that one finds in pernicious anemia. It is the rare case of sprue in which neurologic complica-

tions occur, and we have never seen any neurologic involvement in patients with sprue receiving folic acid.

It would appear from these findings that sprue, in contrast to pernicious anemia, is more nearly a deficiency of folic acid, but that some cases of sprue require factors in addition to folic acid for hematopoietic maintenance over a long period of time.

The next of these anemias is nutritional macrocytic anemia—and here again either folic acid or vitamin B-12 may be effective.

The response of one patient with nutritional macrocytic anemia to a single injection of vitamin B-12 was as follows: there was a reticulocyte response and the red cells increased from around 2,500,000 to around 4,000,000 and then, after a month or so, up to almost 5,000,000, on vitamin B-12 the patient was maintained at 5,000,000. This same patient previously had responded to folic acid. Therapy had been discontinued and she had been permitted to relapse without therapy. Then she was treated with vitamin B-12 and responded. In this individual we have an example of a response to either agent.

It is unwise to make too dogmatic a statement about nutritional macrocytic anemia because of the small number of cases that have been reported and the limited number of cases we have seen at Vanderbilt. It is obvious, however, that remissions may be initiated by either folic acid or vitamin B-12.

Pernicious anemia of pregnancy is something different, however. It has long been recognized that pernicious anemia of pregnancy is not true pernicious anemia. Dr. Carl Moore reported the early cases of pernicious anemia of pregnancy which responded to

folic acid. Since that time a great many people have had similar experience. This may be illustrated by the following example from our series:

One patient came in with approximately 1,000,000 red cells and was treated with folic acid. Therapy resulted in a reticulocytosis up to 50 per cent or so and a very satisfactory hematopoiesis and maintenance of the patient for about three months. We stopped therapy and she has maintained her red cell count at around 4,000,000 or 4,500,000 since that time. She has not been pregnant since.

Another case illustrates the fact that patients with pernicious anemia of pregnancy do not respond to vitamin B-12. This patient became so critically ill during a period of treatment with vitamin B-12 that it was necessary to give her some transfusions; following transfusion she was given folic acid, with excellent reticulocytosis and very good erythropoiesis. She has been maintained since then on folic acid.

As far as I know no one has reported a case of pernicious anemia of pregnancy which has responded to vitamin B-12.

I think this brings up the whole question of folic acid and its relation to the reproductive process. We might digress here for just a moment.

If one recalls the initial work on folic acid, or what we now call folic acid, it pretty much started with Dr. Lucy Wills' studies which were designed to answer the question as to why pregnant women in India developed macrocytic anemia. In order to study this question, Dr. Wills designed diets for monkeys, patterned after the diets of her patients. The monkeys developed an

anemia and leukopenia and eventually died. These monkeys were not protected by administration of potent anti-pernicious anemia liver extracts.

Independently, a year or so later, Dr. Paul Day, at Arkansas, made a similar finding, and eventually it became evident that folic acid and this factor which is necessary for monkeys, and which Dr. Day called vitamin M, were identical—or at least one can certainly say that folic acid has vitamin M activity.

Inasmuch as folic acid seems to be the effective hematopoietic vitamin, and inasmuch as there has grown up a term "Wills factor" for a substance in crude liver extract which is effective in so-called macrocytic anemia or pernicious anemia of pregnancy, it would seem to me that we are justified, at this moment at least, in assuming that the Wills factor is identical with folic acid.

Other experimental studies in animals are interesting in view of the specific nature of folic acid in pernicious anemia of pregnancy. Dr. Roy Hertz, some years ago, showed that the growth of the oviduct of chicks, under the artificial stimulation of stilbestrol, was greatly decreased in folic acid deficiency. He later showed that this growth could be inhibited by one of the folic acid antagonists.

Shortly after Hertz's work, Cerecedo found folic acid to be one of the factors necessary for successful lactation in mice, and, in rats, Evans confirmed the necessity of folic acid for lactation.

Evans then extended these findings, with a demonstration that folic acid is necessary for the gestation process itself; that is, when female rats, placed on a diet which would render them folic acid deficient

(actually this diet had one of the sulfonamides in it), became pregnant they would resorb up to about 44 per cent of the fetuses. Of the young that were born, fewer survived, and of course lactation was poorer. This defect could be prevented by supplementing the diet with folic acid.

Finally, Dr. Hogan found that with a certain strain of rats on a purified diet, some 2 per cent of the newborn young had hydrocephalus unless the diet were supplemented with folic acid.

It does appear, then, that folic acid is used somewhere in the reproductive process. There is other work than that which I have cited, indicating that folic acid requirements of the pregnant rat are considerably higher than those of non-pregnant female rats.

Megaloblastic anemia of infancy shows a variable response to the two hematopoietic agents under discussion. It appears from our experiences and those reported in the literature to date, that all of the cases of megaloblastic anemia which have been treated with folic acid have responded to folic acid. Only a portion of those cases which have been treated with vitamin B-12 have responded.

The mortality from some of the series of megaloblastic anemia under liver extract therapy ran as high as 30 per cent. It is my personal feeling, therefore, that we should consider megaloblastic anemia as a disease which should be treated with folic acid rather than tried on vitamin B-12 and then treated with folic acid, if vitamin B-12 fails.

I would like to say one or two words about the development of megaloblastic anemia. It is very hard

at this stage for any of us to say that megaloblastic anemia is a simple dietary deficiency disease. I don't think we know why infants develop megaloblastic anemia. However, there is some work on its pathogenesis that has been reported, at least in meetings, which ties in with some of our interests. Dr. Charles May of Minnesota has emphasized the frequency of occurrence of megaloblastic anemia in infants who at the same time have scurvy or at least a history of exceedingly low intakes of vitamin C. Most of these infants have been on one or another prepared formula, and their anemia has responded to folic acid.

In attempting to reproduce this anemia, Dr. May has placed monkeys on diets patterned after the preparations used to feed the infants, and has found that he can get the anemia if the monkey is deprived of vitamin C as well as folic acid. If he gives the monkey vitamin C the animal does not become severely anemic.

This seems to explain some earlier studies which were done at Arkansas. Dr. Day, in his early work on vitamin M deficiency in the monkey, produced profoundly anemic animals. In those days he was using 1 to 2 grams of orange a day as a source of vitamin C. Then, when vitamin C became available, this was given along with some other synthetic vitamins that became available about the same time, and the anemia was much more difficult to produce in the monkeys. It was much less severe. None of us could explain this finding until Dr. May explained it for us.

There are other places where vitamin C and folic acid seem to overlap. In chickens, it has been reported, from Wisconsin, that vitamin C has a folic acid-sparing action. The chicken, of course, does not ordinarily require vitamin C, but on a low folic acid diet the

blood level can be better maintained by giving vitamin C along with folic acid than by giving the small quantities of folic acid alone to the chicken.

Some of the studies from our laboratory have been concerned with the metabolic overlap between vitamin C and folic acid. You will recall that scorbutic guinea pigs, as well as premature infants, when given large amounts of tyrosine, will excrete in their urine abnormal metabolic products of tyrosine. We maintained guinea pigs on a diet lacking in ascorbic acid but containing some folic acid. When ascorbic acid was administered, there was immediate disappearance of the abnormal metabolic products of tyrosine in the urine, with their return when ascorbic acid was withdrawn. When folic acid was administered, there was almost complete disappearance of these abnormal products in the urine. When folic acid was stopped, there was a return of these products in the urine.

Pyridoxine failed to prevent the excretion of these abnormal metabolic products. Vitamin B-12 failed to prevent it, and liver extract failed to prevent it under the conditions of our experiment. Pantothenic acid likewise failed to alter this defect.

Pteroyltriglutamate prevents these abnormal metabolic products from appearing in the urine of scorbutic guinea pigs. Here, there is some crossing over of folic acid and vitamin C. However, in this instance, there does not appear to be any crossing over between vitamin B-12 and vitamin C.

I think we are justified in concluding that these two families of B vitamins, that is, the two hematopoietic vitamins, folic acid and B-12, have related effects in man, but that there are diseases which respond much more specifically to one than to the other. Pernicious

anemia is at one end of the spectrum and responds more specifically and more completely to vitamin B-12, with the pernicious anemia of pregnancy at the other end, responding specifically to folic acid.

The whole problem of the interrelationships of these substances and other metabolites is not a simple one, and I believe that in our thinking about them clinically we must always keep before us the fact that there are these innumerable interrelationships, that there are effects other than hematopoietic effects to be studied, and finally, and importantly, that both of these factors are vitamins which are necessary for a great variety of species, and certainly have profound effects in man and are necessary under highly specialized conditions.

## Discussion

DR. HORWITT: This morning I was asked whether or not the polyneuropathy that appeared in some of the patients in our first project could have been associated with a vitamin B-12 deficiency.

I am not very fast on my feet, so I had to sit down for a few hours before I could get the answer. We actually have the answer.

Our second project used the same basal diet. To that diet we added everything but vitamin B-12, because B-12 was the only synthetic vitamin not available at the time. No polyneuropathy appeared in the second project, so we can say that our diet, which was something of a cross between a synthetic and a natural diet, did not produce polyneuropathy because of any vitamin B-12 deficiency.

I would like to ask Dr. Darby if he thinks it possible to associate the antioxidant action of ascorbic

acid with its possible effects in protecting folic acid, either through direct action or through some other compounds.

DR. DARBY: I can't give you an answer to the question, Dr. Horwitt. I might say that we did get mechanistic enough to wonder if ascorbic acid might protect vitamin B-12 if vitamin B-12 were given orally. I am sure several other people did also, after the reports last year that B-12 in assay methods could be protected by vitamin C. We didn't get any positive results. It doesn't protect vitamin B-12 when vitamin B-12 is given orally.

I should like to ask a question: Does anyone here know of any case of pernicious anemia of pregnancy which has responded to vitamin B-12?

AUDIENCE REPLY: No.

DR. DARBY: I certainly don't. I wondered if there were contrary experiences elsewhere.

DR. KAPLAN: In the use of the B-12, are you using it in crystalline form, or concentrated form? Is there any difference in action or reaction?

DR. DARBY: We are using nothing but crystalline B-12. We started early using a crystalline B-12, the Merck product as a matter of fact, and we have been very hesitant to use any other product because one of the purposes of our studies is to get at the minimum effective dose and the minimum requirements.

Inasmuch as the concentrates are assayed microbiologically, I don't think one can feel as confident

that a concentrate has exactly so many micrograms of B-12 in it as one can with a crystalline preparation. There doesn't appear to be any great difference between the activity of the good concentrates and the crystalline preparations.

DR. PIONNI: I would like to ask Dr. Darby whether he feels that the anemia of scruvy is actually due to vitamin C deficiency alone? There is probably a protein deficiency associated with it in anemic animals.

I would like to know if folic acid is not involved in that type of anemia?

DR. DARBY: I think Dr. Vilter could answer that question better than I. He has had much more experience with the anemia of scurvy than I have had.

DR. VILTER: It is still a controversial point, I can tell you what I believe.

I believe that vitamin C is essential for normal blood formation, and that one can find a good many patients with severe scurvy, who have normocytic or slightly macrocytic anemias, who will respond to vitamin C and to that alone.

I think there is also a relationship, as has been pointed out, between vitamin C and folic acid. In the scorbutic patient there are bound to be multiple deficiencies. It is the interaction of a great many factors that results in the anemia, vitamin C having something to do with it (perhaps not all), folic acid probably having something to do with it, and perhaps other things of which we are not aware at the present time.

I think vitamin C does have a specific effect.

# IRON METABOLISM AND HYPOCHROMIC ANEMIA

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Iron metabolism has been so extensively studied during the past five or six decades that it comes as a surprise to many people that there are as many unsolved problems in the field as there are. It seems that the more information one gets about iron, the more confusing the whole subject becomes. Iron serves not only in oxygen transport, as a component of the hemoglobin molecule, but also as a part of the molecule in various enzymes—for instance, the cytochromes; there is still much to be learned in that area.

I thought this afternoon I would like to review the present state of our information about iron metabolism, and try to relate that to our concepts of the pathogenesis of iron deficiency anemia.

Naturally, iron has to be taken into the body, usually by way of the gastrointestinal tract, and then transported by the blood stream to organs of storage and utilization. It would seem, therefore, that the logical way to proceed with such a discussion would be to discuss iron absorption first, then transportation, and finally storage and utilization; but there are practical reasons why it is better to reverse that procedure and start with iron utilization as the first of the things to be discussed.

Iron is required for cellular enzymes, as a part of myoglobin, as a part of hemoglobin; but techniques for the study of iron utilization have been devised only for

the synthesis of hemoglobin, and we will, therefore, confine our attention to iron utilization for this purpose. That can best be studied by using radioactive iron, injecting radioactive iron intravenously in relatively small tracer doses, usually as ferrous ascorbate, and then following, over a period of days, the appearance of the radioactive iron in the circulating blood as newly synthesized hemoglobin.

When normal adult males are given between 9 and 18 mg. of radioactive iron as ferrous ascorbate intravenously there is a rapid appearance of radioactive iron in the peripheral blood, so that within six days' time approximately 60 per cent of the injected iron has appeared in the peripheral blood. At the end of about two weeks, somewhere between 70 and 90 per cent of the injected radioactive iron is circulating as newly synthesized hemoglobin.

It came as a great surprise that injected radioactive iron appeared so rapidly in the peripheral blood as newly synthesized hemoglobin. One can detect hemoglobin containing radioactive iron within a period of four to six hours after the iron is injected. The completeness with which normal subjects utilize the radioactive iron was a second surprise.

In individuals with hypochromic or iron deficiency anemia, there is a more rapid and a more complete utilization, so that roughly 100 per cent of the iron injected is utilized. By contrast, if one injects radioactive iron intravenously into individuals with hypoplastic anemia, who are not making red cells at a normal rate, very little of the iron is utilized. Most of it goes to the storage depots.

The next thing this technique tells us about iron metabolism concerns iron storage.

Most people who have studied iron storage extensively believe that iron is stored largely as ferritin, an iron protein complex, and as hemosiderin.

If one has 5,000 cc of total circulating blood, and has to destroy and remake 0.8 per cent of the total blood volume each day, then one has to make about 40 cc of blood per day. 40 cc of blood per day contains somewhere in the neighborhood of 20 mg. of iron. Over a period of six days, then, one would use only 120 mg. of iron. If the 10 to 15 mg. of radioactive iron had been equally distributed throughout the storehouse of roughly 1 to 2 grams of iron, then one would have expected that, when only 100 to 120 mg. of iron had been used in the first six days, much less than 60 per cent would have appeared in the peripheral blood.

The fact that such a large percentage of the injected iron is utilized has given rise to the concept that there is a labile, physiologically active iron pool, which is called the metabolically active iron pool. This concept has been developed independently by Wintrobe, Ross, Finch and by us. The metabolically active iron pool seems to be in equilibrium with the various storage forms of iron. It is as if the iron stores were a bottle of milk with cream layered on top; when iron is delivered to the iron stores it is first added to the cream on top, the metabolically active iron pool. If it is not utilized, then it diffuses through the rest of the stores. The small amount of iron in the metabolically active iron pool is drawn upon for the day's needs before the ferritin and hemosiderin are called upon. This is very schematic; but there really does seem to be a small amount of metabolically active iron which is used each day for body needs. Whatever is not used probably is stored in various storehouses.

I want to emphasize again that these studies demonstrate that radioactive iron is utilized very promptly for hemoglobin synthesis. It is used relatively completely, giving rise to this concept of a metabolically active iron pool. Lastly, this technique gives us a rough way of measuring the rate of red cell formation and hemoglobin synthesis.

From this I want to turn now to a consideration of iron absorption.

Iron absorption has been studied in a number of different ways, but probably the best technique is that devised or first suggested by Paul Hahn, again using radioactive iron. This technique is a combination of measuring absorption and utilization. That is why I wanted to discuss utilization first.

What one does is to give radioactive iron by mouth and then measure the per cent of the oral dose of radioactive iron which appears in the peripheral blood as circulating hemoglobin, and assume that that amount is equivalent to the amount of the dose absorbed.

In an experiment on an anemic dog, made anemic by chronic hemorrhage, 1 mg. of ferric iron was given per kilogram of body weight. The dog absorbed 45 per cent of the oral dose. The experiment was repeated after the level in the peripheral blood became constant, and this time the same dose of iron was given, but as the ferrous salt, and there was an additional rise in the amount of radioactive iron in the peripheral blood equivalent to about 40 per cent of the dose.

In this study on an anemic dog it appears as if ferric and ferrous iron are equally well absorbed. That usually happens in dogs; once in a while one finds an ani-

mal that absorbs ferrous iron better than ferric iron, but the rule in our laboratory has been that dogs are able to absorb both forms equally well. That is something which Dr. Whipple claimed for a number of years.

By great contrast, however, is the experience in human subjects, wherein if one gives ferric chloride and then ferrous chloride, one regularly finds that much more of the ferrous salt is absorbed than of the ferric. When 2 mg. of iron as ferric chloride were given to a subject with hypochromic anemia, 6 per cent of the oral dose was absorbed. The same amount of iron was repeated as ferrous chloride, and 26 per cent of the dose was absorbed.

With this technique, then, we have been able to demonstrate conclusively that some animals are able to absorb ferric as well as ferrous iron, but human subjects absorb ferrous better than ferric iron. That suggests but does not prove that, in the human subject, iron is absorbed only as ferrous iron, and any ferric iron taken into the oral cavity must be reduced in the intestinal tract to the ferrous form before it can be absorbed.

In a similar study done on myself some years ago, 2 per cent of the dose of ferric iron was absorbed and 11 per cent of the ferrous iron was assimilated.

In hypochromic anemia, quite regularly one gets absorption that tops 20 per cent of the total dose, whereas, in normal individuals, the amount of iron absorbed is always in the neighborhood of 10 per cent or less. There is no doubt about the fact that individuals who are iron deficient absorb iron more readily and more efficiently than do normal persons.

This has given rise to the concept that the intestinal mucosa is an important regulator of iron metabolism. It occurred to Dr. Hahn that perhaps the intestinal mucosa is an all-important factor, absorbing iron when it is needed and rejecting it when it is not needed.

Some support for Dr. Hahn's concept is offered by the following experiment. A man with hypochromic anemia absorbed 57 per cent of an oral test dose—a tremendous absorption. Then he was given 1,345 mg. of inert iron intravenously, in one dose, as colloidal ferric hydroxide. That was calculated to be enough iron to raise his hemoglobin level from 9 grams up to normal. Then, a short time later, the iron absorption curve was repeated. This time only 24 per cent of the oral dose was absorbed. Since he was less iron deficient, he absorbed less iron.

Dr. Grannick has suggested that iron in food must be reduced to the ferrous form, that it then is absorbed by the mucosal cells of the gastrointestinal tract and combined with a protein called apoferritin to form ferritin. When all the apoferritin has been combined with iron to form ferritin, no additional amounts of iron will be absorbed by the mucosal cells until they have had a chance to give up some of their iron to the blood stream, where the iron circulates in plasma as a combination of iron plus beta-1 globulin, this, of course, goes on to the various storage depots.

Dr. Grannick's concept, then, is that the mucosal cells tend to regulate iron absorption by limiting the amount of iron which may be combined with apoferritin, the amount of apoferritin formed being the deciding factor.

A truth which Dr. Hahn expressed is that iron deficient subjects absorb iron more effectively than do

normal subjects. This has been overstated, however, to imply that the person who doesn't need iron rejects all iron and absorbs practically none.

We gave a person with pernicious anemia radioactive iron by mouth, again 1 mg. per kilogram of body weight. If the observation had been stopped before liver therapy was given, as happened in the other cases recorded in the literature, one would have gotten the impression that practically no iron was absorbed from the gastrointestinal tract, and that the intestinal mucosa had rejected the iron because it didn't need it. But studies on iron utilization taught us that sometimes iron could be stored in tissues and not utilized; so we gave this and several other individuals liver extract; no more radioactive iron at all was given; and, as the hemoglobin value went up, the radioactive iron in the peripheral blood also rose to a value of about 22 per cent, indicating that this individual, who was anemic but certainly not iron deficient, had absorbed 22 per cent of the test dose.

If, in addition to measuring the amount of radioiron that appears in the blood, you measure the amount which you can recover in the feces during the first seventy-two or ninety-six hours after the oral dose, you will find that, in iron deficient subjects, the amount of iron that you recover in the feces plus that which you find in blood as circulating hemoglobin totals 100 per cent, plus or minus 10 per cent, which is the error of the method. With this additional measurement, in other subjects we found evidence that iron was retained by the body even though it wasn't used for hemoglobin synthesis.

In a group of normal individuals the amounts of iron which actually appeared in the peripheral blood

varied from 2 to 11 per cent. In every instance an appreciable amount of iron was absorbed, not as much as by the individuals with iron deficiency, to be sure, but an appreciable amount. Furthermore, we found considerable absorption (as measured by the difference between the oral dose and fecal recovery) in two individuals with hypoplastic anemia, who put out practically no radioactive iron in the peripheral blood because they weren't making hemoglobin and each of whom had received more than 100 transfusions and must have had in excess of 20 grams of iron stored in the tissues. One of these on one occasion absorbed 14 per cent, and on another occasion 27 per cent of the test dose. The other absorbed 15 per cent of the test dose.

In other words, individuals who are normal, or individuals who are anemic and who have plenty of iron in their storehouses, still do absorb appreciable amounts of iron. The factors which control iron absorption from the intestinal tract must still be considered a mystery. The factor of iron need is only one of the things of importance.

From here I want to go to the all-important subject of iron excretion. Again a truth has been overstated considerably through confusion in the literature.

It has been stated that the iron requirement of individuals during the early years of life is high because they are growing rapidly and their total circulating hemoglobin is increasing, that during adolescence the requirement for the female rises and stays high during the active sexual period, but that the iron requirement for the male falls as he reaches the age of 21 or 22 years and actually hits zero.

This is an over-statement of a principle, because, obviously, the iron requirement for the normal male cannot hit zero. Iron is a component of all the cells of the body. There is desquamation from our body all the time. We lose iron in our hair, in our fingernails, and there has to be at least that much iron lost. The question is, how much iron, in addition, is lost in the gastro-intestinal tract?

In order to answer that, we gave radioactive iron to a number of normal individuals, and studied their iron excretion over a period of 140 days, in periods of five days at a time. This long period was taken because we found that the small tracer doses of iron that we gave were utilized largely for hemoglobin synthesis. By prolonging our observations beyond 120 days, we hoped to be able to catch any great outpouring of radioactive iron which might occur when these red cells which contained the radio-iron were destroyed.

Actually there was no large excretion at any time. The highest value found was in the neighborhood of 0.03 per cent of the dose per day. The average value was approximately 0.01 per cent per day for the 140 days.

How can one use this figure to calculate the total amount of iron excreted? Here is where one has to make a number of assumptions which may not be correct.

The total circulating hemoglobin in one individual was estimated to be about 1,200 grams. The amount of iron in the hemoglobin was approximately 4 grams. The amount of radioactive iron was approximately 5 mgs. The ratio of inert to radioactive iron, then, was 800:1. The amount of iron excreted daily amounted

to 0.01 per cent. 5.6 mg. of iron were injected as the tracer dose, so that the amount of radioactive iron excreted daily amounted to 0.00056 mg. That figure multiplied by 800, to correct for the ratio of inert to radioactive iron in the peripheral blood, gives a value of 0.45 mg. of iron excreted in the feces per day.

For another subject, 0.35 mg. was excreted in the feces per day; for another, 0.40 mg. A normal woman working in the laboratory excreted 0.40 mg. per day, if our calculation is valid. A woman with hypochromic anemia excreted in the feces only 0.059 mg. per day.

The iron in the feces could come from one of three sources. It could come merely from the desquamation of the epithelium of the gastrointestinal tract; it could represent an active excretory function; it could come from bile for it has been established that there is an increased amount of iron in bile.

In order to satisfy ourselves about the source of the iron in the feces, hemolytic anemia was produced in a dog with phenylhydrazine and the radioactive iron excreted in the feces was measured. During the period of rapid fall in hemoglobin value, the amount of radioactive iron excreted in the feces was comparatively large. It gradually fell off as the dog recovered from the hemolysis.

That is understandable. It has been established that the amount of iron in bile increases during a hemolytic episode. We know that, if you give iron by mouth, it is not 100 per cent absorbed. It is reasonable, then, to think that iron dumped into the gastrointestinal tract through bile will not be 100 per cent absorbed, and that there will be a slight increase in excretion.

In summary, we found that, for normal individuals, the fecal excretion of iron per day is somewhere in the neighborhood of 0.3 to 0.5 mg.; for hypochromic anemia the excretion is much less, and for individuals (at least the occasional individual) with a hemolytic anemia, the excretion of iron in the feces per day may be quite high. Also, the amount of iron lost in the feces per day is comparatively small.

Last March all concepts of iron metabolism were seriously disturbed when a paper by Dr. Mitchell, at Champaigne, appeared, stating that, in a group of boys whom he was following on a long metabolic study, he had obtained evidence that the amount of iron excreted in sweat, under comfortable conditions, might be as much as 6 mg. per day.

That is a very disturbing figure. Suppose we try to figure how much food iron would have to be taken in to compensate for that loss. If 50 per cent of all the iron in the food were ionizable, one would have to multiply that 6 mg. by 2. If his absorption from the intestinal tract were 50 per cent complete, which is a very high figure, one would have to multiply by 2 again; and the minimum amount of iron that such a normal male could get by on would be 24 mg. in his diet per day.

None of us takes that much. Also, if so much iron were lost in the sweat, one would expect iron deficiency anemia to be much more prevalent in hot regions than in dry; and that isn't necessarily true. Iron deficiency anemia is probably most frequent in Britain and in the Scandinavian countries.

We have had an opportunity to complete three studies on the excretion of iron in the sweat, using radio-

active iron. The radioactive iron was given intravenously as ferrous ascorbate, and the sweat was collected over various two-hour periods. In one study, the volume of sweat amounted to 1,010, 1,050 and 600 cc for the two-hour collection period on the first, the seventh, and the thirty-sixth day. The radioactive iron recovered in that amount of sweat, in terms of the per cent of the dose, amounted to 0.0007, 0.002 and 0.002 per cent.

If one assumes that the individual lost sweat at the same rate—and that is fantastic, because it takes rather severe conditions to make one lose 1,000 cc of sweat in a two-hour period—but, if one make that assumption, the twenty-four-hour excretory rate would be 0.0084 per cent of the radioactive iron on the first day, 0.024 per cent on the seventh day, and 0.024 per cent on the thirty-sixth day.

The first collection was made in the two-hour period immediately following the injection. The urine during these two hours had approximately fifty times as much radioactive iron as did the sweat. The urine volume was considerably less than 1,000 cc, as you might expect.

Here we have to make another assumption, if we are going to find out what this means in terms of excretion of iron in sweat per day. If the total body iron is assumed to be 5 grams, and if the same per cent of total body iron is excreted in sweat as is excreted as radioactive iron, then the maximum amount of iron which could be excreted would be 0.42 mg., 1.2 mg. and 1.2 mg. per twenty-four hours—and these conditions are stacked tremendously on the high side. It seems to me that the chances are that the amount of iron lost in sweat per day is certainly less than 0.5 mg.

The other two studies which have been completed show the same tendency, except that I should point out that I have selected for presentation here the highest values of the three.

For calculating the iron requirement of an individual, I believe that the daily excretion by the male in feces would probably be less than 0.5 mg., and all other forms of excretion—sweat, desquamation of cells, loss of hair, loss of fingernails, toenails, and so on would not make up more than another 0.5 mg. The iron requirement of the adult normal male would probably be less than 1 mg. per day. In the female, we have to add to that an average menstrual loss of somewhere in the neighborhood of 1 mg. per day to bring the normal female requirement to between 1.5 and 2 mg. of iron.

If 20 per cent of ionized food iron is absorbed and 50 per cent of the iron in food is ionizable, then a male would have to take in 1 x 5 x 2, or something less than 10 mg. a day to remain in balance, whereas a woman would have to take in between 15 and 20 mg. a day.

These are very rough figures. A normal woman probably absorbs a little more than 20 per cent of the small amount of ionizable iron present in food, to reduce these figures down to approximately those figures which have been given by the Food and Nutrition Board, which I think were surprisingly good estimates.

One of the interesting things to comment on is the effect of blood donation on iron requirement—something that isn't given as much attention as it deserves.

500 cc of blood contains 200 to 250 mg. of iron. The average loss per day, if one gives one transfusion a year, is 0.57 to 0.7 mg. of iron. In other words, that

just about doubles one's iron requirement. If one gives five transfusions a year, which is done very frequently, the iron required to replace just the iron lost in the transfusion would be 2.85 to 3.5 mg. per day. If you multiply this to compensate for the facts that all the food iron is not ionizable and that absorption is not complete, you come up with the finding that individuals who give five or six transfusions per year run a serious danger of becoming iron deficient, unless their diets are supplemented with elemental iron.

Here is a brief summary of all I have said about iron metabolism.

Ingested iron goes into the stomach, where it comes into contact with free HCL, which helps begin the reducing process to the ferrous state. Iron is absorbed in the small intestine, probably best in the duodenum and progressively less well as one proceeds distally.

Iron is transported in plasma, in combination with a beta-1 globulin. The transport iron in plasma is in equilibrium with a number of factors; the ferritin and hemosiderin in the storage depots, the iron that goes to hemoglobin, the iron that goes to tissues for the formation of cellular enzymes and the small amount excreted.

Once the bone marrow gets iron, it forms hemoglobin and passes it on to the circulating blood. When the red cells are destroyed at the end of 120 days, the iron released for the most part goes back into the body pool, to be used all over again.

The greatest iron loss is from hemorrhage, but it is an over-simplification to say that the body has no capacity to excrete iron, as I hope I have demonstrated.

What does all this mean in terms of the pathogenesis of iron deficiency anemia? People still talk about

idiopathic iron deficiency anemia. There is no such thing. Iron deficiency anemia can be caused only by an iron deficiency, and iron deficiency can result in only one of two ways—either by an inadequate intake or assimilation of iron, probably the chief cause of iron deficiency anemia in childhood, or by iron loss through blood loss, generally associated with a poor diet or poor absorption from the intestinal tract because of diarrhea or achlorhydria.

The payoff question is this: Is it possible for a normal adult male, who has no blood loss whatever, to develop an iron deficiency, if he has a very poor diet? Theoretically, there can be enough loss of iron from the gastrointestinal tract and from sweat so that, over a period of years, an adult male, who loses no iron by hemorrhage but is on a very poor diet or absorbs iron very poorly from his gastrointestinal tract, ought to be able to develop a nutritional iron deficiency. So far that has not been observed and, in the adult, it is still true that hemorrhage is the *sine qua non* for the development of iron deficiency; and one must have hemorrhage, it can be normal menstrual loss, in association with other factors leading to deficient assimilation.

## Discussion

DR. KINGSLEY M. STEVENS (University of Chicago): I don't feel that the fact that you get a little difference between the absorption of radioactive iron in patients with anemia and a normal patient can really tell you very much about the net change in the two.

Here you measure the uptake of iron by the blood, but what we are interested in is the net transfer across the intestinal mucosa.

While in both the anemic and non-anemic there could be the same uptake, excretion into the intestinal tract could be greater in the normal. By following radioactive iron concentration in the intestine; one couldn't tell this, since that which was absorbed would go into the metabolic pool and be so diluted as to make differences very small.

DR. CARL V. MOORE: If you give iron intravenously to a normal or an anemic subject, either one, and none by mouth at all, you don't find any increased loss of iron in the stool during the first twenty-four hours, which would suggest that the excretion of iron through the intestinal wall must be very small.

We have tried awfully hard to get evidence that there is active excretion of iron into the colon or into the small intestine. We would like very much to get such evidence. All of the experiments we have done, and those everybody else I know of have done, indicate that, if this happens at all, it is mighty little.

DR. DARBY: Dr. Moore, I would like to have you comment on the significance for the human of so-called available iron, as measured by animal studies.

DR. MOORE: Dr. Darby is referring to the fact that we all talk about available iron in food, or the ionizable iron in food; that percentage of food iron in such simple formation that the hydrochloric acid in the stomach can ionize it, and which can be absorbed.

That has been measured either by chemical methods or by actually feeding the foodstuffs to iron deficient animals and measuring utilization. That was all done originally by Dr. Elvehjem and his associates.

A number of people have criticized these studies, but it seems to me that, until one gets more definitive information, one should accept them. I don't know of any data to indicate they are not correct.

The thing that might be done—and Dr. Darby and I have talked about this—is to try to grow spinach, lettuce and other things in a pure medium to which the only iron added is radioactive iron. If one fed such foods, one could really measure the percentage of available iron.

DR. DARBY: It seems from your studies on dogs that what is true for one species doesn't necessarily carry over to man.

DR. MOORE: If it were ferric iron in food, normal man might not be able to absorb it as well as the normal animal.

DR. COWGILL: Isn't it true that some of the feeding experiments have not yielded the same results as the chemical method? Some foods have been tested and rated by the biological test, and then rated by the simple chemical analysis and the results haven't been the same. This suggests that any rating has to be taken with a grain of salt. We don't know all we would like to know about this.

DR. KARK: In India, in troops of equal physique, strength and development, there were definite differences in the hemoglobin content of blood from rice-eating soldiers and wheat-eating soldiers. The iron content of the rice-eaters' diet was about 25 mg. per day, but the rice-eating soldiers had statistically lower hemo-

globin levels than the wheat-eaters who ingested only 12 mg. of iron per day.

Recently, I believe Dr. Finch has shown a definite relationship between the absorption of iron and the phosphorus content of the diet. Perhaps this is the crucial situation in relation to iron requirements and this may also explain the differences in hemoglobin levels which are found in rice and wheat eating Indians.

DR. MOORE: I think there is no doubt whatever about the probability that Finch's results are correct. The more phosphate there is in the diet, the more phosphate can hook onto iron to form insoluble iron phosphate and decrease absorption.

In our studies of iron absorption we have used artificial techniques because we have given radioactive iron in the fasting state and have kept individuals fasting for periods of four to five hours after the dose, to try to eliminate complicating factors. When one is dealing with absorption of food iron, unquestionably phosphate is one of the important factors—but it must be only one.

I didn't mention in this brief review Dr. Finch's very interesting studies which bear on the question of what regulates iron absorption from the gut. He became interested in the work of the Gillmans, in Johannesburg, who pointed out that a number of the natives who ate mostly corn developed hemachromotosis by the time they reached the age of twenty-five or thirty, and at death had as much as 20 to 25 grams of iron stored in their livers. He followed up that observation and fed rats a diet of hominy grits and found that the animals stored perfectly tremendous amounts of iron. By adding an extra amount of phosphate he could cut down

on the absorption of iron. Here again is another example of animals, with iron stores above normal, continuing to absorb large amounts of iron, under an artificial set of circumstances.

CHAIRMAN BESSEY: Are there any other questions? We know many other examples of such differences between species. Compare the human and the rat in connection with calcium absorption; there are tremendous differences. The ratio of absorption surface of the small intestine of the rat to body weight is very much larger than the corresponding ratio for man. Therefore the rat more efficiently absorbs calcium.

# THE PROBLEM OF THE EVALUATION OF OBESITY AND ITS SIGNIFICANCE

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Questions of obesity, overweight and fatness, have been occasioning much interest lately, for a variety of reasons. One important reason is that insurance and actuarial experts are increasingly insistent that obesity is a very important factor in their mortality statistics. The fact is that the only nutritional "defect" which occasions a separate life rating table, is that having to do with excess of calories, or obesity.

There is no point in reviewing here the evidence on the increased mortality associated with overweight; it is very impressive, and, as the insurance statistics improve, it becomes more impressive. It seems to be associated with increased mortality due to cardio-vascular diseases, diabetes and degenerative states.

There is evidence that, if accidents were removed from the mortality tables—and accidents are quite important in the United States as a cause of death—the obesity penalty would be even greater. I take it from this that there is some suggestion that fat people don't move around so fast and, therefore, they are not so apt to get killed in accidents.

We are involved all the time, in our everyday converse, our everyday business, in basic descriptions of individuals, and certainly one of the most important things that comes up is fatness. When we examine anybody we note something about sex, something about

age, something about general size or height, something about fatness. But we have no agreed-upon definition for fatness and we frequently find that we are talking about different subjects when we talk about overweight or obesity. There is an unfortunate tendency to confuse the one with the other. Clinical appraisals of fatness or of the general nutritional state frequently seem to be dependent not on overweight or on any general estimate of fatness at all, but rather on the appearance of the subcutaneous fat. In a recent clinical paper on this subject in children, it was stated flatly that obesity *is* subcutaneous fat.

These uncertainties cause misconceptions, disagreements, and finally a lack of quantitative character in our definitions.

I believe it is correct to say that a complete dissociation between general caloric nutriture and specific nutriture is rare. I mean by this that people who are suffering from major nutritional defects of a specific character frequently also show some peculiarity or abnormality in regard to their general or caloric nutriture.

Practically all of the information we have on obesity in the modern literature is gained from classifying people according to height-weight tables. I remind you of one or two things about height-weight tables: They are all based on the general idea that the average is somehow good. The general procedure is to take a group of people, throw out those who are obviously diseased, measure the heights and weights of those who remain and are called non-diseased and take the average; the result is the "standard". That is a very questionable procedure, of course. In actual practice it has been even worse because, with a few

exceptions, unrepresentative population samples have been used to set up these so-called standards for height and weight.

In the United States, practically the only tables in common use are based on the medico-actuarial studies of 1912, in which records from insurance applicants were analysed in this fashion. The data came from limited areas of the United States as well as from restricted samples of the population within those areas.

This means that we have in the United States (and this is true also of most of the rest of the world) no unbiased information about the total population beyond the age of thirty, even defining the simple average as good or ideal or standard.

In the actual construction of the medico-actuarial tables, it is surprising to find that there has been no differentiation between the mean and the mode, in spite of the fact that the material is very pronouncedly skewed; this means that the standards are biased toward greater obesity than the actual middle of the population. The tables are numerically inadequate.

Another point is that there is no allowance made for such technical questions as diurnal variation in height. People very frequently will show changes of a half to three-quarters of an inch in height from morning to afternoon.

Finally, the question of the body frame or body type has not received adequate attention, although it has been discussed very often. There are many references to heavy and light frames and to different body types, without any acceptable basis for quantitative placement of individuals in these categories.

The standard height-weight tables indicate nothing but the arithmetical averages for certain people in certain parts of the United States in the years preceding 1912.

I have been talking about relative weight for height; presumably we should allow for the lateral dimensions of the body framework also, but for this there is no agreed upon basis.

Another measure which has been used is the relative abdominal girth, the waistline circumference per unit height or chest dimensions. Various elaborate analyses, all bad so far as I know, have been made on these bases.

The subcutaneous fat is of particular interest because, if man is similar to some other mammals, about half of his total extractable fat is present in the subcutaneous layer. If one could get an estimate of the subcutaneous fat, even in linear dimensions, it should be very useful.

Two ways of doing this have been suggested. First, there is mechanical measurement of the skin fold. This has been rather extensively used with school children in certain parts of Scandinavia and, more recently, in England. A few studies have been made in the United States. It has been used very little for adults anywhere, although I suppose the great majority of clinicians use it subconsciously in practice. They take hold of people while they are putting them in a good psychological relation with their doctor, they feel their arms and backs, and get an idea about fatness in this way.

Another method is x-ray. With the limbs, it is possible with the x-ray to delineate quite easily the skin and the subcutaneous fat. The anatomists who have studied these questions assure us that the true skin

is practically the same thickness in everybody and that differences, then, in the skin folds and of total "skin" thickness represent differences in fat.

At Minnesota we have worked rather extensively with body density. Before us, Captain Behnke and some of the group at the Naval Medical Research Laboratory were interested in this for some years. The basis of the use of body density for estimating obesity is simple. Human body fat has a specific gravity of about 0.91. The muscular and glandular tissues have densities on the order of 1.1 to 1.2, and the bone mineral has a density of about 2.0.

The allowance for the contribution of bone is not as much of a problem as one might think. Again we lack basic data, but apparently in normal man only about 4 per cent of his total body weight is made up of bone mineral. Even if there are considerable variations in the bone mineral in different persons of the same height, our final calculations would not be too much in error because the total bone mineral is so small. If we increase the bone mineral of the body by 50 per cent, we add only 2 per cent to the total body weight.

Body density can be estimated, as we have done in many thousands of determinations, by weighing the person completely submerged in water and making an appropriate allowance for the residual air in the lungs. At the present time we are working, with some hope of success, on a similar arrangement using air as the ambient fluid and measuring air pressure in a fixed space.

Measurement of the total body water has been suggested recently as a means of estimating obesity, on the basis that the total body water seems to be related not

to the fat but to the muscular and more active tissue of the body. Therefore, if one had a measure of total body water and total weight, one might be able to estimate how much fat and how much more active tissue there is in the body. Dr. Murray Steele and his associates, as well as others, have been interested in this; they have attempted to measure total body water by the use of deuterium oxide and antipyrine.

Another method which might be considered for estimating obesity is blood composition. This was thought of many years ago but was abandoned. It might be looked into again, however; it is conceivable that the concentration of some substances in the blood may be related to the amount or concentration of fat in the total tissues.

Basal metabolism has been neglected in this regard. Basal metabolism presumably reflects the metabolism of the active tissues; fat is, in terms of energy metabolism, a very inactive tissue. But there are difficulties here; for example, the variable activity of the thyroid gland would have to be considered.

Finally, there are the most direct measures which can seldom be applied to man. Direct chemical analysis of the whole body has been made only in two or three adult bodies in the whole of the history of biology, once recently at the University of Illinois, at Urbana, and the "normal" man there, I think, was less than perfectly normal. He died at the age of thirty-five, in congestive heart failure. Presumably he had been inactive for quite a long time and had congestive failure; there certainly must have been some abnormality of hydration as well.

When normal man is starved so that he loses about 15 per cent of his body weight, there is no important change in his total extracellular fluid. There is a very large change in fat, and a sizable but not so large change in the active tissue.

As starvation is carried on until the total weight loss is 25 per cent, the active tissue shrinks a great deal. The fluid and blood spaces remain about the same. The bone is unchanged. Practically all the change takes place in the fat and active tissues. Therefore, 25 per cent weight loss represents far more than that, in terms of the loss of active tissue and of fat. The loss of active tissue is more like 30 or 35 per cent and, the loss of fat is of the order of 75 to 80 per cent.

When measurements are made over a long period of time, during which weight is lost and then regained, the importance of paying attention to the question of fat in the total weight picture becomes obvious.

We have studied starvation and recovery carried out to 58 weeks of recovery; there were 24 weeks of semi-starvation, and 12 weeks of pre-starvation control observations. As recovery takes place, body weight is regained and may surpass the original weight, but there is a tendency to lay down more fat than active tissue. Eventually, after many months, the extra fat is lost and the composition of the body gets back to normal. Simply recording weight would not have told us this, and would not have provided us with the basis for the analysis of the metabolic problems which are associated.

Such considerations suggest that the metabolism of the body must be related to the mass and character of

the tissue which is doing the metabolizing; the important relationship is not to the surface of the body but rather to the mass of the body.

When we computed a starved person's basal metabolism according to body mass, we found only about a 15 per cent reduction in metabolism as starvation proceeded; this is to be compared to 40 per cent, using the body surface computation. When we made a further analysis, allowing for the fact that bone mineral obviously does not participate in energy metabolism, that fat participates very little, and that the extracellular fluid space certainly doesn't have a high metabolism, we found only about a 5 per cent reduction in the rate of metabolism of the living tissue of the body. We arrived at quite a different picture of the changing metabolism, then, by subdividing the body into its major components.

These are all reasons for paying particular attention to the estimate of fat.

Over the last year and a half to two years we have been trying to see to what extent different measures of fatness in the body are related to one another. In a study of 159 normal young men, of all degrees of fatness, we found that the relative weight is correlated with the specific gravity by a coefficient of —0.728. It has the same correlation with the abdominal skin fold. The specific gravity is correlated with the abdominal skin fold to an even higher degree,—0.824. The important thing is that relative weight, according to standard height-weight tables, is only correlated with true fatness to the extent indicated. A large amount of uncorrelated fatness goes undetected by the use of the relative weight.

We studied middle-aged men, from the ages of forty-five to fifty-five, and found much the same picture, except that the correlations were lower. The correlation between relative weight and specific gravity was only —0.65.

The coefficient of correlation between the relative weight and the abdominal skin fold was 0.45. That is not an impressive correlation, to be sure; it is very significant statistically, but it does not provide a useful basis for numerical prediction of fat.

When young men and middle-aged men are considered together; relative weight is correlated only to the extent of a coefficient of –0.24 with specific gravity, and of 0.475 with the abdominal skin fold. Specific gravity is correlated quite highly (inversely) with the thickness of the abdominal skin fold. It appears that, with a mixed age group, relative weight is less useful than it is within a limited age range, and that there is a closer relation between the skin fold on the abdomen and specific gravity than between the latter and relative body weight.

Obviously we are interested in knowing how obesity is related to other characteristics. In this regard we have studied the cholesterol in the blood. With the same group of 159 normal young men, the correlation between relative weight and serum cholesterol is 0.192. There is a correlation, not very great but significant, between the amount of cholesterol in the blood and relative weight. We found the same kind of correlation with cholesterol with specific gravity and with the thickness of the abdominal skin. No matter how we measure or attempt to estimate the fatness of young men, we find that fatness is related to the serum cholesterol level in a positive manner.

On the other hand, if we consider the intake of cholesterol, the relation between the intake and these other items is insignificant throughout.

With the older group there is much the same picture, except that here the only measure of fatness which is correlated significantly with cholesterol in the blood is specific gravity. Specific gravity correlates to the extent of –0.179, the 1 per cent level of significance is –0.171. This is a highly significant correlation.

We know, then, that in these normal men there is a relation between obesity and blood cholesterol and that the obesity measure which gives the best relation is fatness as estimated by density.

Let us take up one other point that people are interested in. That is the basal blood pressure, as recorded in at least four independent observations on these 383 normal men.

In the younger men there seems to be a slight but rather questionable relation between the systolic and diastolic pressure and the per cent of standard weight. Much the same is true of the older men, with perhaps a little more apparent relation in the fatter men.

Another point that is of interest is that there is not the slightest difference between the young men and the old men with regard to resting blood pressure at equal fatness.

If, instead of standard weight, we consider obesity as determined by specific gravity, there is no doubt that, both with systolic and diastolic blood pressure and both with young men and old men, there is a definite relation between blood pressure and obesity. These were normal men examined under basal conditions. Finally,

it should be noted that, under such conditions, the range of blood pressure differences between individuals is much smaller than usually thought of or as indicated in the literature.

In normal men, then, specific gravity as a measure of fatness, or fatness as estimated from specific gravity, is certainly related to blood pressure in the basal state. It is a more pronounced relation than we would imagine by looking at the body weight itself.

I think we must agree that obesity, as fatness, and overweight are of both theoretical and practical importance. However, they are definitely not the same, and may have opposite physiological significance. The well trained football player, who is six feet tall and who weighs 200 lbs., will actually have less than the normal absolute amount of fat in the body, as far as we can make out. Nonetheless he is "overweight" by the tables. Metabolically there is a great difference between this kind of overweight and that of simple fatness.

We must insist that there are no good data of any kind, not even good theoretical analyses of the question as to the meaning or use of standard or ideal weights, for the evaluation of obesity and relative fatness in this regard.

At the present time all indications are that body density measurements are very good, but they are certainly not practical for widespread application. We have found it possible to measure weights under water of a good many hundred men—not only students, but also some hundreds of business men—but we do find it a little difficult to work with middle-aged women and some of our men in this under-water arrangement. We

hope that body density, as estimated by the aerodensitometer, which is rather a complicated gadget, at least in my laboratory at the present time, may prove a more useful tool.

In using heights and weights at the present time, we are in great need of an agreed-upon objective system for rating the frame or type of body so that this can be taken into account.

For general application, however, I think skin fold studies seem to have the greatest possibility. They can be made very readily. The caliper problem we have found to be not difficult and we have made a number in my laboratory. The questions of compression of the skin, and so on, do not seem to be too troublesome.

We hope that within a few years we may begin to remove some of the major question marks about the measurement and standardization of obesity, so that the problem of its relation to the basic questions of morbidity and mortality and the life habitus can be brought under attack.

### Discussion

DR. I. PAT BRONSTEIN (University of Illinois, Chicago): I would like to ask Dr. Keys a question: In the young group you had a relation between serum cholesterol and obesity. In that group, was the cholesterol intake measured?

Also, I would like to know what you considered to be important variations in serum cholesterol.

DR. KEYS: These charts are all taken from a long-range study on a group of men who have been cooperating over some years with us; we get extremely good cooperation, by the way.

We have estimated the cholesterol intake in these men by various means—by questionnaires and by sitting down and actually eating a meal with them—they come and spend the day at least once a year for such purposes.

Cholesterol intake is not difficult to estimate, roughly at least. Whether eggs are eaten for breakfast, or are not eaten for breakfast, determines whether a person is at one end or the other of the scale. You can't go very far wrong in placing them at least in broad categories of low, medium and high.

We make five divisions that way. The persons in the lowest cholesterol intake category will never have more than about 1.5 gram or a little more of cholesterol per week. Persons in the highest categories will never have less than 3.5 to 4 grams per week.

Variations in individuals with regard to cholesterol intake are quite large. However, they are consistent in the same individual, as we have found by following them over a period of three years. The relation, then, between the value of cholesterol in the blood and fatness, and the absence of a relation between cholesterol intake and fatness, is taken care of by the method of partial correlations. The tables show there is a relation between serum cholesterol and obesity but the correlation is not high.

# COMMENTS ON SOME PROBLEMS OF OBESITY

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The subject of obesity has a great many phases. It has economic aspects, such as the cost of food, clothing, furniture, and restrictions of the main opportunities for earning a living. It has social aspects. To some, the inability to wear nice clothing is important, and to one girl of eighteen, who weighed 190 lbs., her inability to secure dancing partners was very important.

It also has medical aspects. Heavy bodies wear down weak legs and weak feet. Cardiac disorders are aggravated. There also are disorders, such as hypertension and diabetes, which may be regarded as physiological strain phenomena. All these and many other aspects are important; but, in this discussion, we are going to consider obesity from the point of view of its metabolism.

Before we enter into a discussion of the strictly metabolic features, I would like to spend a moment on three of the common stumbling blocks in the discussion of obesity; about 75 per cent of the doctors get into trouble on this subject and practically all the laity have misunderstandings on these points.

The first point: the obese are small eaters. There is a great deal of confusion regarding mass of food as contrasted with the energy content of that mass. In a study of eight hospitalized patients, the average intake on a freely selected diet was 2,570 calories. The maximum intake was 3,690 calories, the minimum 1,450.

The patients lost an average of 2 lbs. in four days; the amount of food they were eating was not adequate for them to hold their weight.

We took another series of six patients, put them on fixed diets and watched what happened to their weights. These six patients averaged an intake of 2,500 calories a day. They lost an average of 1.4 kilograms in twelve days, or about 120 grams per day per person. If you assume that 120 grams of weight represents about 900 calories, these patients, sitting around the ward doing nothing, consumed about 3,500 calories a day.

A couple more simple illustrations: One patient weighed 106 kilograms and, in six days, lost 1.4 kilograms on a 2,500 calorie diet, 230 grams a day. A 95-kilogram woman lost 130 grams a day in nine days, on a 2,500 calorie diet. A woman of 151 kilograms lost 1 kilogram in nine days, on a 3,000 calorie diet. I bring these observations to your attention to emphasize the fact that people who are large eat large quantities of food.

The second point that is commonly misunderstood is the matter of obese people getting more out of their food than ordinary people do. What are our indices of the efficiency of digestion? They are very poor. We can take the weight of the feces and assume that feces represent food residues, which we all know is not true, but that's the best we can do.

We compared the feces weight to the food weight on diets running from 1,300 to 2,700 calories and found an average ratio of 8.3 per cent, a range of from 11 to 4.5, not a very good correlation. Comparing feces solids with food solids, you get a better comparison. With diets running from 1,300 to 2,700 calories, we

found that the average ratio was 5.8 per cent; that is, the dry weight of the feces represented 5.8 per cent of the dry weight of the food intake. We had another series in which we compared diets of 2,000 to 5,000 calories. There again the ratio was 4.7 to 7.5. We have not been able to demonstrate any significant variation in digestive efficiency of people with normal gastrointestinal tracts, regardless of their sizes or weights.

Thirdly, we have the matter of glands. People look at a patient and see that he or she is a little unusual in proportion, and say, "It's a glandular disorder."

Endocrine processes regulate metabolism. They don't initiate any new processes, and they can work only on material which has been supplied through the process of nutrition. So, we regard obesity as a problem in nutrition.

There are certain endocrine changes which are often associated with obesity. In one group there are the strain phenomena such as diabetes. In diabetes, the carbohydrate-handling capacity of the body is strained. High glucose tolerance curves appear in the obese state and return to normal when the obesity is removed. I don't think any of us would say that diabetes is a cause of obesity. Hypertension is another type of strain phenomenon, perhaps endocrine in nature. Hypertension does not cause obesity.

When obesity is associated with pituitary and hypothalamic lesions, there is always hyperalimentation.

Ovarian and menstrual problems occur particularly in young girls. These are not the cause of obesity—they are the result of obesity, and, in most cases, can be corrected by correcting the obesity.

Endocrine processes are regulatory processes. When the individual is subjected to strain from forced nutrition, he may show certain abnormal endocrine patterns, but the endocrine disorders don't cause the obesity. The body changes and the endocrine changes are the results of the physiologic strains associated with obesity or with the hyperalimentation that precedes the obesity.

A number of theses that have been suggested from time to time, with reference to obesity, suggest that the laws of conservation of mass and of conservation of energy are not applicable to human metabolism. We have collected evidence to show that they are applicable. It is important to keep in mind that storage fat is not just a static material laid down and left in place for a year or two or ten but is constantly being replaced. In one series of experiments published by Buchanan and Hastings, one-half of the total fat deposits of the animals was replaced in a matter of seven days. That is a pretty rapid turnover for material which we ordinarily consider as dead storage. These bodies of ours are undergoing a constant turnover, and the rate is far greater than most of us have appreciated in the past.

In a mass balance experiment we try to trace the mass of intake and the mass of output and see if we can find a relationship between the mass of intake and the mass of output and whatever changes we observe in the residual mass, which is the body.

Let us consider what happens in a normal individual. I have the data for one 62 kilogram girl whom we studied for thirty-seven days. She had 117.2 kilograms of food and water. Her weight loss was 0.8 kg. The urine and feces were 65.9 kg. and 3.1 kg., and insensible

perspiration amounted to 47.5 kg. The total intake was 118 kg. and the total output was 116.5 kg. In thirty-seven days, she turned over a mass of material which was almost twice her body weight. The weight of the urine was over 50 per cent of the total and was greater than her body weight. The insensible perspiration was a little less than her body weight. The daily turnover corresponded to about 5 per cent of her average body mass.

We have some figures for an obese person, collected over a period of 176 days while she was kept on a rigid diet of about 550 calories. Her intake figure was 731 kilograms. Her weight loss was 47 kilograms; urine, 403; feces, 68; insensible perspiration, 265; total output, 732 kilograms. To turn those figures into ordinary terminology, it means that this woman turned over, in 176 days, six-sevenths of a ton of material. Her average turnover was 4,400 grams or 10 pounds a day. There is no evidence here of a small intake.

I am going to pass over the matter of the determination of food and water and some of these other things, and will take up two of the difficult points in mass balance; first of all, the estimation of insensible perspiration. It is not a simple thing to do. Our calculations are based on a paper published by Root and Benedict back in 1926, in which they demonstrated a definite relationship between the weight of the insensible perspiration in the basal state and the basal metabolism. From their data we developed a formula, and then we made a gross assumption that, if the basal insensible perspiration paralleled the basal metabolism, the total of one paralleled the total of the other. That may not be correct for people who are out running around. For

people in a hospital, in a guinea pig cage, as we had these people, we think the assumption is justifiable. Certainly, the results of the calculations we have made make us feel that it is not too far wrong.

The next difficulty is the estimation of body weight loss. That sounds like it should be a very simple thing to do, but it isn't.

First of all, what is your body weight? Those of you who have had experience with these beam balances that are very beautiful mechanisms, and also very expensive and difficult to operate, know it is almost impossible to determine a person's weight on a beam balance. A person is pretty much the same as a little vial of calcium chloride, on a beam balance. It is difficult to weigh calcium chloride on a beam balance because it continues to change its weight.

It is perfectly obvious that we are dealing with a constantly changing weight. We have this constant flux of intake and output. Sometimes in the day it is greater than at other times, but we also have the basic insensible weight loss of about 1 gram per minute, anyway.

We have tken the standard weight as the weight of an individual at seven o'clock in the morning, before he has had any food or water and after he has emptied the bladder. If you take weights under those standard conditions, which are the best we can conceive of, what will you get if you take a normal individual and weigh him day after day? You won't get the same weight every morning. One girl of normal weight was weighed for thirty-seven days under these standard conditions, and on only one observation was her weight the same

on two successive days. The average daily weight change was 320 grams. That same phenomenon is also present in the obese.

We have data to suggest that this weight shift is due to a shift in the storage of water. We also have data to suggest that there is no relation at all between the water which you actually drink in the course of twenty-four hours and the amount of water which stays in the body.

These minor water swings are found in normal people and they are also found in the obese. There are other types of water swings. There is the water swing which comes with menstruation. As all of you know, it is not at all unusual for women to gain 2 or 3 lbs. at the time of their periods. In the treatment of obesity, it is important to recognize that this water storage phenomenon may be exaggerated. There are two types of swing: First, there is the very moderate swing which we see in most obese patients in the first week of dieting. Patients will drop anywhere from 2 to 5 lbs. more than you would expect them to drop on the calculated degree of nutrition.

An example of this is a woman who weighed 194 kilograms. She dropped 4.3 kilograms in three days. In the next seven days she dropped a total of 8.4 kilograms. At that time she was 4 kilograms below the weight which we would predict for her.

What is the cause of this peculiar phenomenon? I can't tell you, but there are two components of it; one is the fact that when you start a patient on a reduction diet he or she will always go into negative nitrogen balance for a few days. You will recall that protein material ties up a lot of water, as contrasted with fat.

Then there is the phenomenon of the high ketogenic-antiketogenic ratio of the metabolized mixtures. The ratio in some of our diets ran from 3 to 3.5, and it is known that there is a dehydrating effect of ketogenic factors of that magnitude.

One of the most troublesome phenomena in the treatment of fat people, particularly the grossly obese, is the second type of giant water swing. I am going to describe one characteristic swing for you. Obviously I am picking out one of our best to illustrate the point.

The patient had been dieting for three months, and in the third month she had been on her prediction curve; that is, her daily weight loss was very close to what we were expecting her to lose. On the 8th of July she was on the prediction curve, and the 9th of July she lost 3.6 kilograms. She went 3 kilograms below her prediction curve. On that particular occasion she put out 3900 cc of urine. There was no diarrhea.

She went for another eleven days, during which she lost practically no weight. The daily up-and-down phenomenon that I described applies all through this, of course. On the 7th of August she was back on her prediction curve, and then she continued to gain weight. On the 24th of August she was 3 kilograms over her predicted weight; beginning on the 26th of August she lost 3.6 kilograms in a matter of two days. At that time she had diarrhea as well as an increased amount of urine; but she got back to her prediction curve.

Clinically, this is a very difficult phenomenon to deal with. If you try to keep a patient on a strict reduction diet, and that patient sees her weight going up

and not down, day after day, you have a difficult time continuing the program.

It is important to keep in mind that one of these water swings may last six weeks. Some reported studies seem to have been stopped in the middle of the swing and weren't carried on until the patient had a readjustment of body weight.

We have a real problem in estimating probable tissue loss. In a normal individual it is not too difficult. We use the average of five successive days' weight at the beginning and at the end of the observation period. If you use a sufficiently long period of observation, you get away from the effect of the small swings.

In the matter of energy balance, we have the same dynamic equilibrium to consider; the body represents not only a mass of particles but also the energy content of the particles. The components of an energy balance study are pretty much the same as those for mass balance. You have your basic intake factors and the basic output factors. Under intake we have both food and weight loss. On the other side we have basal metabolism, the specific dynamic action, the work fraction, the non-work fraction and the energy content of the urine and feces.

Now let's consider the matter of the energy equivalent of the weight loss. That requires a little consideration. We have already pointed out the difficulty in deciding what the true weight loss is. We have agreed that we are going to use the five-day average weight as probably representing body weight at a given time.

The kind of tissue lost from the body greatly influences the calculation. Protein tissue changes result in

rapid weight losses for a relatively low calorie loss. The caloric value of protein is four. Protein tissue is dry protein plus water and water is three times the weight of protein, giving us the rough figure that one gram of weight corresponds to one calorie, when dealing with protein tissue.

Fat is a different type of material. It has a high caloric content as far as the heat equivalent per gram of dry fat is concerned (9.3 calories), and fat carries very little water with it. It is a little difficult to find out how much water is in fat tissue.

We have looked up the literature and have made some studies of our own, taking masses of fat and determining the water content. In fat, under ordinary conditions, the water content varies between 15 and 30 per cent, in contrast to 300 per cent in the case of protein. When you lose one gram of weight in fat you are losing 9 calories.

In general, our experience has been that rapid weight losses not due to water shifts are associated with lost protein, and that weight losses due to fat loss are slow.

Just a point about basal metabolism: I would like to call your attention to the fact that the term "basal metabolism" refers to heat production. The form in which that heat production is expressed in most of the literature gives an incorrect impression of the amount of heat produced by obese people. The usual form is b.m.r. It has been known for many years that the b.m.r., the basal metabolic rate of the obese, is normal, plus or minus 10. That was pointed out in about 1919, at least.

If you express heat production in its simpler form of calories per hour, you get the proper impression of the amount of heat that these people are producing. One series of obese patients produced heat at the average rate of 69 to 79 calories per hour. For comparable people of the same age, sex and height, and so on, the calories would be about 55 to 70.

One patient, who weighed 426 lbs., had a basal metabolism of 99 calories per hour. She was putting out 2,380 calories per day basal, and yet her b.m.r. was −1, or something like that.

As a measure of excessive heat production in the obese, we compare their heat production to that which these patients themselves would have if they were of normal weight. We studied a group of five patients with an average weight of 222 lbs.; average calories per hour, 71; average surface, 2 meters; average b.m.r., −3. Comparing the average heat production to that calculated by using the so-called ideal weight, it is found that these patients were 72 per cent overweight, that the excess surface was 26 per cent, and that the b.m.r., on the basis of ideal weight, is plus 23 per cent.

The patient, who weighed 426 lbs., produced 99 calories per hour; her b.m.r. was plus 1. On the basis of her ideal surface, the b.m.r. is plus 56. If you want to use the b.m.r. convention, I think it desirable to substitute ideal weight for observed weight, to get a measure of the increase in heat production in these people.

You have heard and have read that the specific dynamic action is out of order in patients who are obese. Properly speaking, this refers to the total heat effect of

a meal, and not to the effect of a meal at any given moment of observation.

Benedict and a number of his co-workers, many years ago, demonstrated that most of the heat effect of a meal is exhibited in eight hours. We tried a series of observations, using a 610 calorie meal which produced 57 calories of specific dynamic action in eight hours. About two-thirds of the heat effect appeared in the first four hours.

There are a number of pitfalls in this determination. First is the base line. The base line has a tremendous effect on the resulting calculations. It is very important that you have a well trained subject.

You find in the literature a number of discussions of the negative phase of heat production following a meal. In other words, the heat production an hour or two after a meal is lower than the initial basal heat production. Our experience with that phenomenon is that it is due to an erroneous base line.

This is another field in which the form of expression of your results affects your judgment as to what is going on. There are a great many reports in the literature dealing with single observations of heat production an hour after a meal. If you use the term "b.m.r." you will not get into your calculation the unusual size of the basal heat production in an obese person and the small amount of heat production in a thin person, or an intermediate heat production in a person of normal weight. When you make your calculations you will get the impression that you have a small heat production in the obese and a large heat production in the thin. That is not true.

We had a series of five patients, of normal weight, with an average initial b.m.r. of −6.8. We had six obese patients with +2.2 average, and three thin patients with −9.0. When you calculate the specific dynamic action in the manner I have indicated, the change in the normal was 18 per cent in the first hour, in the obese 14 per cent, and in the thin 21 per cent. It looks as if you are proving the point that there is a difference in the three states. Actually, however, if you look at the number of calories concerned, they are 12, 11, and 13, in the three groups. Our experience with these three types of people has been that there is no difference at all in the way a thin person and a fat person and a person of normal weight respond to food. We feel that the specific dynamic action, the heat effect of the food, is determined by the type of food and not by the body that eats it.

We have the matter of work and non-work factors. They are very difficult to estimate under any conditions. Our patients had no work fraction because they were sitting around, doing nothing. The non-work fraction is susceptible to estimation and, for our mass balance experiments, we use the food intake and the heat equivalent of the weight change as representing total metabolism.

In estimating total metabolism for energy balance, we conduct the best type of mass balance we can, and regard the difference between the measurable intake and measurable output as insensible perspiration.

In general you can construct an energy balance, and you can construct a mass balance within 4 or 5 per cent of balancing the intake and the output.

We can summarize this part by saying that we can't find that the obese behave any differently from normal people as far as mass and energy are concerned, and we have found no evidence of any significant deviations from the laws of mass and energy in either state.

Now I would like to spend a moment on the matter of treatment of obese patients. We use the thesis that fat represents a store of energy caused by an excess of intake over output. The fat can be removed by producing an excess of output over intake, provided the metabolism is maintained at normal levels in all other aspects. That, of course, is the "catch". It brings us to the matter of essential foods and what they are.

Obviously, protein is essential; how much is essential is a matter of debate. Nobody knows the optimum level of nitrogen metabolism. We consider 1 gram of protein per kilogram of ideal weight to be adequate; that is a safe figure.

The amount of carbohydrate one needs is an interesting question. As you know, carbohydrate is mixed up in protein metabolism, and it is mixed up in fat metabolism, and there is a certain amount of carbohydrate that probably has to be metabolized in its own individual manner. Just how much carbohydrate is essential to maintain normal metabolism is not susceptible to any theoretical demonstration that I know of. Practically, if you keep your patient in nitrogen equilibrium you, presumably, are giving adequate carbohydrate. 40 grams of carbohydrate per day will keep any of these patients in nitrogen equilibrium; as a matter of actual practice, certainly in office work, we often run that figure up to 80. Certainly 80 grams a day is adequate for any metabolic needs.

That leaves the matter of the source of most of the energy, namely, the fat desposits in the body tissue. There is the matter of essential fatty acids. I cannot give you any information on that point. I have tried one or two experiments, supplying the so-called essential fatty acids in some of our restricted diets, but I can't see that any of our patients, even those on our most restricted diets, ever get into any of the troubles that are described by the people who write about essential fatty acids.

Now, the matter of the rate of reduction: What can you expect in the line of rate of reduction? You can't expect anything more than the limiting factor of caloric deficit multiplied by time. That is going to be your total weight loss, and the caloric deficit is the regulating factor in how fast that weight is going to go down. Under hospital conditions, we have had patients lose 6 lbs. a week, 380 grams a day, for considerable periods of time. According to our calculations, that corresponds to a deficit over 2,200 calories a day.

In office work, we have never been able to use diets as strict as our hospital diets. The best results we have had have been 4 lbs. a week for twenty-four weeks, which is a pretty good rate. If you can get a pound or two weight loss per week in a person who is just moderately obese, you are doing very well. The rapid rates are obtained on people who weigh 350 and 400 lbs.

There are certain problems in treatment that I want to mention. First of all, cost. Strange as it may seem, a fair number of dispensary patients and poor patients are fat. One reason for this is that carbohydrate is cheap food, and, in trying to get their protein requirements in cheap foods, these people eat a tremendous

amount of carbohydrate. Keep in mind that protein food is costly food, and a high percentage of dispensary patients can't afford it.

Another matter is vitamins. For practical purposes, we use one poly-vitamin capsule a day, using the accepted standards of values, and we have never had any trouble with vitamin deficiencies in our patients.

Another point is the cost of the doctor. Don't forget that, in office practice, the physician's fee is added to the cost of food and vitamins, and the cost of other things. In dispensary practice, perhaps that is not too important.

Then there is the matter of family practices. Where is your patient going to get his meals? Too many doctors sit down in their offices and simply write out diets for patients. They do not consider where the patient is going to get the food. This doesn't apply to obesity any more than it does to diabetes or any of the other problems for which we write diets.

If a person is on a reduction diet and eats his meals at home, he has the constant problem of the cook serving two menus, one menu to the rest of the family and one to the patient; or, if an obese patient happens to be the female of the family, everyone in the family goes on the reduction diet, when she does.

Then there is a fair percentage of patients who take their meals in restaurants. This is particularly true of unmarried men. An unmarried man who has been put on a diet is a very difficult problem, regardless of whether he is obese or a diabetic. Where in the world is he going to get his food?

Those of you who have eaten in restaurants (and I presume some of you have) know the character of the so-called a lá carte meals, or club meals. In general, such meals have too little protein, too much fat, and too much carbohydrate. The outstanding feature of reduction diets, as we use them, is the high protein content, compared to what the patients have been accustomed to eating. Actually, they are not high protein in the sense of the absolute value for protein.

Another of the problems in treatment is the matter of drugs. What drugs are you going to use? As far as the thyroid group of drugs is concerned, we feel that their use is not only psychologically wrong but is physiologically wrong. We are dealing with a nutrition problem, not an endocrine problem. The level of metabolism in these fat people is already high. Even if you could push it higher, there is no point in trying it.

The dinitrophenol group, which was popular about ten years ago, is practically off the market now, and I think it is forbidden. That particular group of materials was very dangerous as well as psychologically wrong.

Then we come to the amphetamine group, which is popular now. I don't think there is any place in the treatment of obesity for any of these drugs.

There is another factor in this practical problem, and that is time. These diets are monotonous. A vast majority of patients who require diets are food addicts; they love food, and in order for them to lose appreciable amounts of weight they have to go on a diet, and they have to stick to it. The monotony of this diet causes a great many people to fall by the wayside.

Another problem that is very important is the matter of water swing. If you try to encourage a patient to stick to a diet when she is in the middle of a water swing that is lasting for two or three weeks, you know what you are up against. A patient has enough difficulty sticking to a diet, just looking at the monotonous food day after day, without having the additional insult of seeing her total weight on the scales going up. That is a morale problem and it is a very difficult one to deal with, I assure you.

We have the personality problem. People who become grossly obese are addicts. They are just as truly food addicts as others are morphine addicts or alcohol addicts. If you are going to get anywhere with these people, you have to change their food habits, and it takes some character in order for any adult to change any kind of habit.

There are certain patients who will cooperate—and those patients, in my opinion, fall into the groups who are frightened or those to whom weight reduction means dollars. There are two real urges for people to go on a diet and stay on it. There are certain social values which you might call dollars. There is a life insurance premium which is definitely dollars. We have many people who go on diets in order to get their premiums reduced. Also, there are certain people who are frightened. Diabetics don't want to take insulin. Hypertensive people are afraid they are going to have strokes. Cardiacs want to do a little more walking around and climbing stairs. You can frighten such people into sticking on a diet, or they can frighten themselves into it.

We have a very low rate of success. Less than 25 per cent of our patients accomplish what we regard as a successful reduction. We feel that a good part of that is due to our definition of success, which means that the patient not only has to get the weight down but has to keep it down. We feel that another reason for our low rate of success is that patients who have enough character and enough "stuff" in them to stick to a reduction diet and make a success of it don't get fat in the first place.

I was going to spend some time talking about future problems for study, but time does not permit it. We haven't solved the problem of obesity by merely pointing out that these people eat a lot and that you can get weight off them by the practical measure of decreasing their intake.

I would like to leave this thesis with you: We have been concerned about obesity from the point of view of why obese people have enormous appetites. Appetite is why people start to eat. I think it is most important to figure out why they don't stop eating. Why is it that a person of normal weight gets a certain feeling that he wants to quit eating?

I am sorry we have had to abbreviate this part of the discussion.

(The meeting adjourned *sine die* at 6:00 p.m.)